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AICE SURVEY OF USSR AIR POLLUTION LITERATURE. VOLUME XVII. A COMPILATION OF TECHNICAL REPORTS (4TH) ON THE BIOLOGICAL EFFECTS AND THE PUBLIC HEALTH ASPECTS OF ATMOSPHERIC POLLUTANTS

M. Y. Nuttonson

American Institute of Crop Ecology

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1972

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Volume XVII

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Edited By

M. Y. Nuttonson

The material presented here is part of a survey of
USSR literature on air pollution
conducted by the Air Pollution Section
AMERICAN INSTITUTE OF CROP ECOLOGY

This survey is being conducted under GRANT R 800878
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OFFICE OF AIR PROGRAMS
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3 CZECHOSLOVAKIA—Agricultural Climatology of Czechoslovakia and Its Agro-Climatic Analogues in North America

4 YUGOSLAVIA—Agricultural Climatology of Yugoslavia and Its Agro-Climatic Analogues in North America

5 GREECE—Ecological Crop Geography of Greece and Its Agro-Climatic Analogues in North America

6 ALBANIA—Ecological Plant Geography of Albania, Its Agricultural Crops and Some North American Climatic Analogues

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11 SWEDEN—Agricultural Climatology of Sweden and Its Agro-Climatic Analogues in North America

12 NORWAY—Ecological Crop Geography of Norway and Its Agro-Climatic Analogues in North America

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16. Abstracts

A collection of papers are presented which deal with the biological effects of low concentrations of ambient chemical toxic substances found in various industrial areas. The papers are titled as follows: 1. Maximum Permissible Concentrations of Noxious Substances in the Atmospheric Air of Populated Areas, 2. Data for a Sanitary Validation of Maximum Permissible Concentrations of Sulfur Dioxide in Combination with Phenol, 3. Data for a Sanitary Basis of the Mean Daily Maximum Permissible Concentration of Nitrogen Dioxide in Atmospheric Air, 4. Experimental Data for a Sanitary Standardization of Low Concentrations of a Mixture of Furfural, Methanol and Hydrolytic Ethanol in Atmospheric Air, 5. Threshold Concentrations of Diketene during Short-Term Inhalation, 6. Experimental Validation of the Mean Daily Maximum Permissible Concentration of Vanadium Pentoxide in Atmospheric Air, 7. Determination of the Threshold of the Reflex Effect of Chloroprene by the Method of Electroencephalography, 8. On the Toxicity of Microconcentrations of Ethylene Oxide, 9. Sanitary Standardization of Alpha-Methylstyrene in Atmospheric Air, 10. Sanitary Validation of the Maximum Permissible Concentration of Divinyl in Atmospheric Air, 11. New Experimental Data for the Validation of the Maximum Permissible Concentration of Hexamethylenediamine in the air of Populated Areas.

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Air Pollution
Biological Detection
Toxicity
Standards
Airborne Wastes
Industrial Wastes
Populations
Sulfur Dioxide
Phenols
Nitrogen Dioxide
Furfurals
Carbinols

Ethanols
Ketenes
Vanadium Oxides
Chloroprene
Electroencephalography
Ethylene Oxide
Styrene
Vinyl Compounds
Heterocyclic Compounds

17b. Identifiers/Open-Ended Terms
USSR
Maximum Permissible Concentration
Alpha-Methylstyrene
Vanadium Pentoxide
Diketene
Divinyl
Hexamethylenediamine

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1972

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PREFACE

The present volume constitutes the fourth* compilation of the USSR technical reports on a number of investigations of the biological effects of specific air pollutants. These investigations have been conducted at various public health institutes and in departments of public health of some of the universities of that country.

The development of industrial chemistry in the USSR has called attention to the toxicological significance of the new chemical air pollutants and made it imperative to investigate their biological effects. It also suggested the need to conduct investigations dealing with the public health implications of the new chemical air pollutants.

The material included in this volume deals with the biological effects of low concentrations of ambient chemical toxic substances such as those found in conjunction with

(1) the metallurgical industry,

(2) metal etching, metal engraving, and autogenous welding,

(3) the hydrolysis plants,

(4) the production of textile materials, wetting agents, plastic solvents, and chloroprene synthetic rubber,

(5) the production of mineral fertilizers, explosives, dyes, pharmaceutical preparations, anticorrosive substances, plasticizers, etc.,

(6) the production of nitric acid, acetoacetic acid and its complex esters, as well as other ethers and esters.

The results of the above studies provide in the USSR a basis for the establishment of a series of new maximum permissible concentrations for new toxic substances in the atmospheric air and constitute the scientific criteria for assessing the degree of air pollution. They also form the foundation for a number of proposed ameliorative sanitation measures.

Some background information on the distribution of the Soviet industry's production machine may be of interest in connection with that country's present and potential pollution problems and investigations. The planned distribution of production in the Soviet Union favors effective exploitation of the natural resources of the USSR, especially in its eastern areas where enormous natural resources are concentrated, and has led to the creation of large industrial centers and complexes of heavy industry in many of the country's economic areas (see page ix). The many diverse climatic conditions of the country and its major economic areas as well as the geographical distribution of the Soviet Union's principal industrial and mining centers and of its principal electric power stations and power systems can be seen from the various maps presented as background material in this volume.

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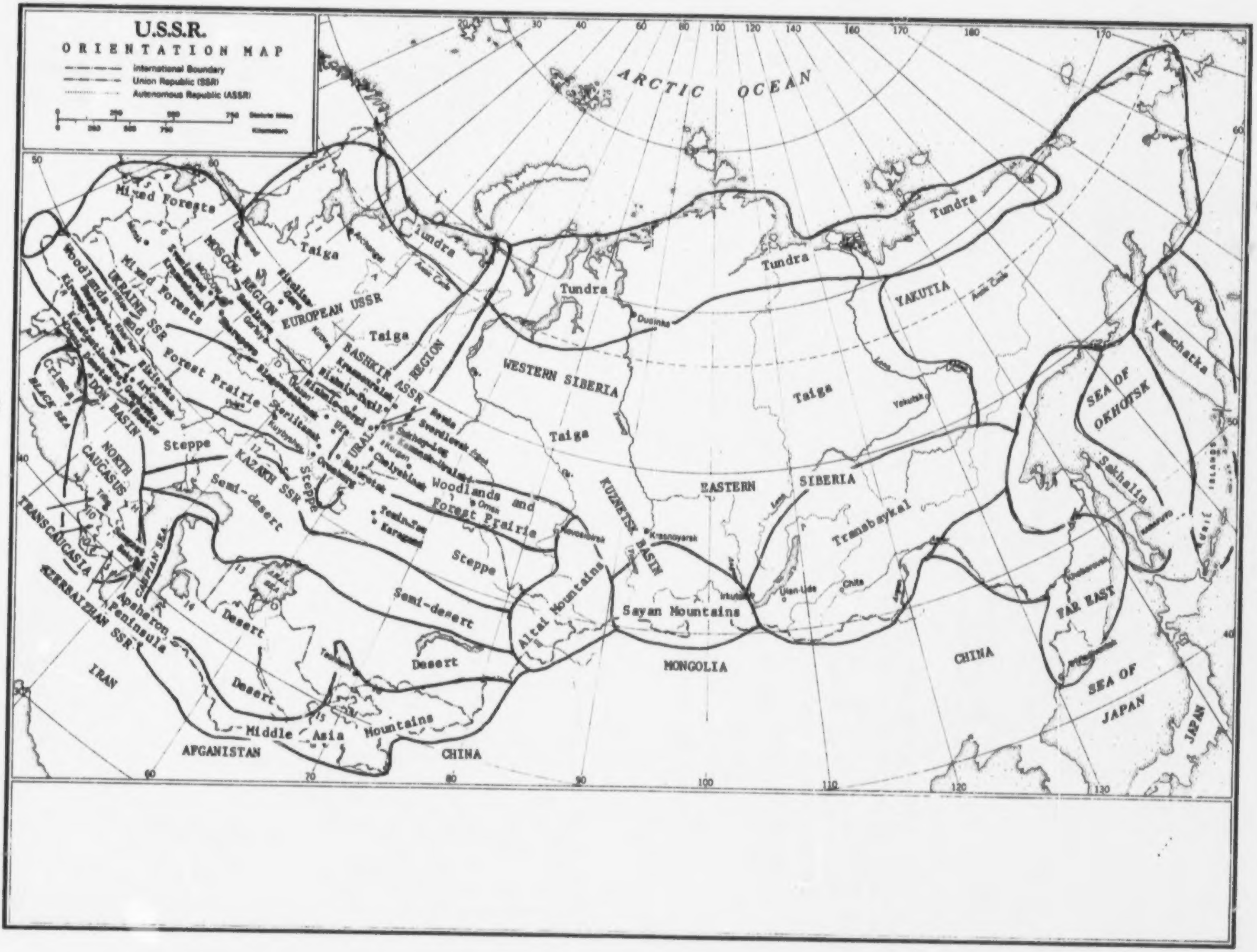
* The first compilation of this nature has been published in Volume VIII, the second in Volume XI, and the third, in Volume XII of the AICE Survey of USSR Air Pollution Literature.

It is hoped that the papers selected for presentation in this volume will be conducive to a better appreciation of some of the air pollution investigations conducted in the USSR. As the editor of this volume I wish to thank my co-workers in the Air Pollution Section of the Institute for their valuable assistance.

M. Y. Nuttonson

September 1972



ADMINISTRATIVE DIVISIONS

S.S.R.

1. R.S.F.S.R
2. Karelo-Finnish S.S.R.
3. Estonian S.S.R.
4. Latvian S.S.R.
5. Lithuanian S.S.R.
6. White Russian S.S.R.
7. Ukrainian S.S.R.
8. Moldavian S.S.R.
9. Georgian S.S.R.
10. Armenian S.S.R.
11. Azerbaydzhan S.S.R.
12. Kazakh S.S.R.
13. Uzbek S.S.R.
14. Turkmen S.S.R.
15. Tadzhik S.S.R.
16. Kirgiz S.S.R.

A.S.S.R.

A. Komi ASSR
B. Udmurtskaya ASSR
C. Mariyskaya ASSR
D. Chuvashskaya ASSR
E. Mordovskaya ASSR
F. Tatarskaya ASSR
G. Bashkirskaya ASSR
H. Dagestanskaya ASSR
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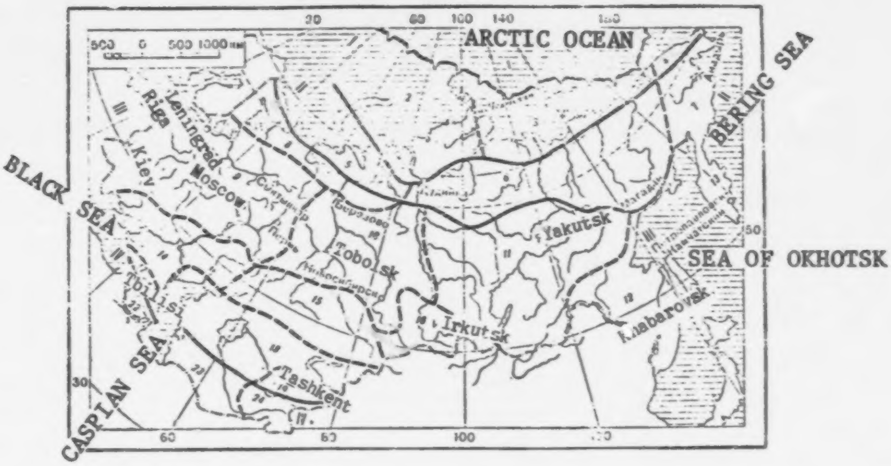
K. Kabardinskaya ASSR
L. Abkhazskaya ASSR
M. Adzharskaya ASSR
N. Nakhichevanskaya ASSR
O. Kara-Kalpakskaya ASSR
P. Buryat-Mongol'skaya ASSR
Q. Yakutskaya ASSR

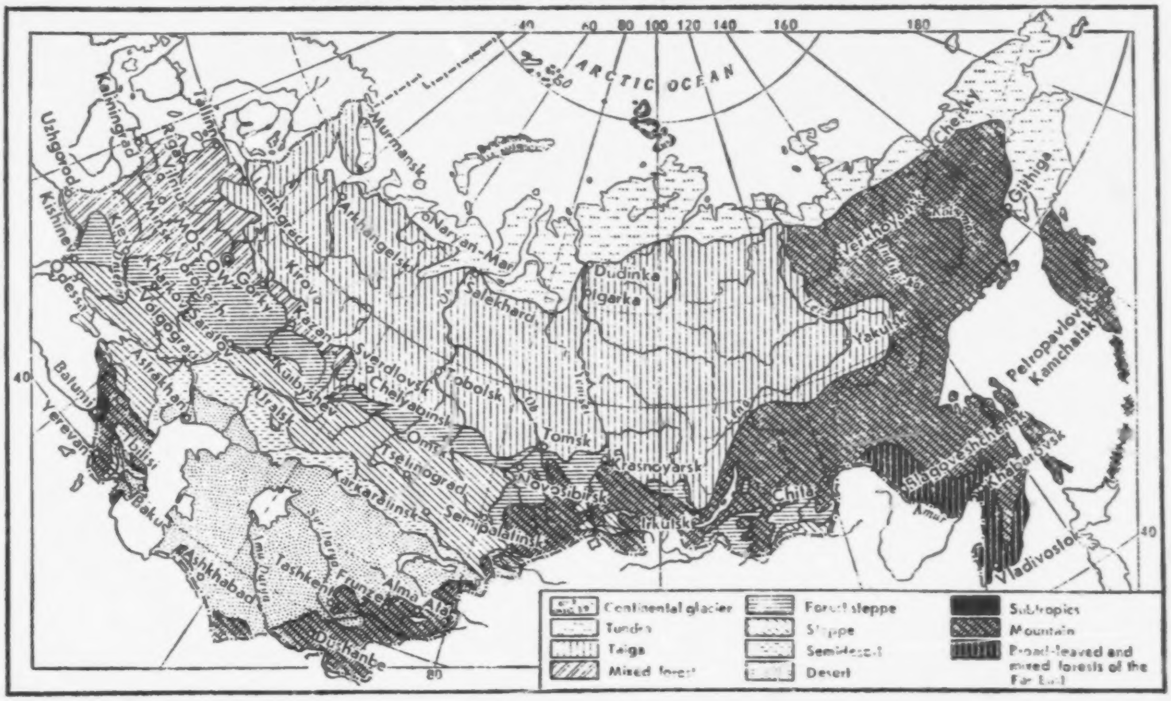
CLIMATIC ZONES AND REGIONS* OF THE USSR



Zones: I-arctic, II-subarctic, III-temperate, IV-subtropical
Regions: 1-polar, 2-Atlantic, 3-East Siberian, 4-Pacific, 5-Atlantic, 6-Siberian, 7-Pacific, 8-Atlantic-arctic, 9-Atlantic-continental forests, 10-continental forests West Siberian, 11-continental forests East Siberian, 12-monsoon forests, 13-Pacific forests, 14-Atlantic-continental steppe, 15-continental steppe West Siberian, 16-mountainous Altay and Sayan, 17-mountainous Northern Caucasus, 18-continental desert Central Asian, 19-mountainous Tyan-Shan, 20-western Transcaucasian, 21-eastern Transcaucasian, 22-mountainous Transcaucasian highlands, 23-desert south-Turanian, 24-mountainous Pamir-Alay

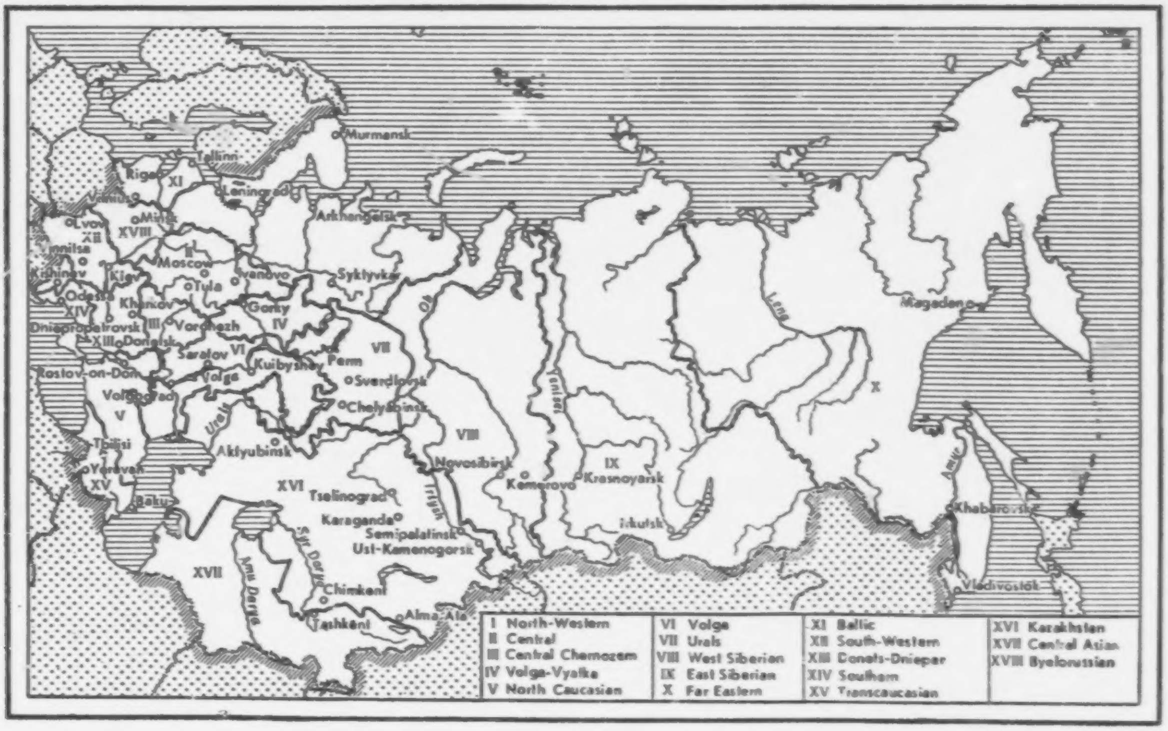
(After B. P. Alisov, "Climate of The USSR", Moscow 1956)

SOIL AND VEGETATION ZONES IN THE U.S.S.R.



(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

MAJOR ECONOMIC AREAS OF THE U.S.S.R.



PLANNED DISTRIBUTION OF INDUSTRIAL PRODUCTION IN ORDER TO BRING IT CLOSER TO RAW MATERIAL AND FUEL SOURCES

An example of the planned distribution of industrial production in the USSR is the creation of large industrial centers and complexes of heavy industry in many of the country's economic areas: the North-West (Kirovsk, Kandalaksha, Vorkuta), the Urals (Magnitogorsk, Chelyabinsk, Nizhny Tagil), Western and Eastern Siberia (Novosibirsk, Novokuznetsk, Kemerovo, Krasnoyarsk, Irkutsk, Bratsk), Kazakhstan (Karaganda, Rudny, Balkhash, Dzhezkazgan).

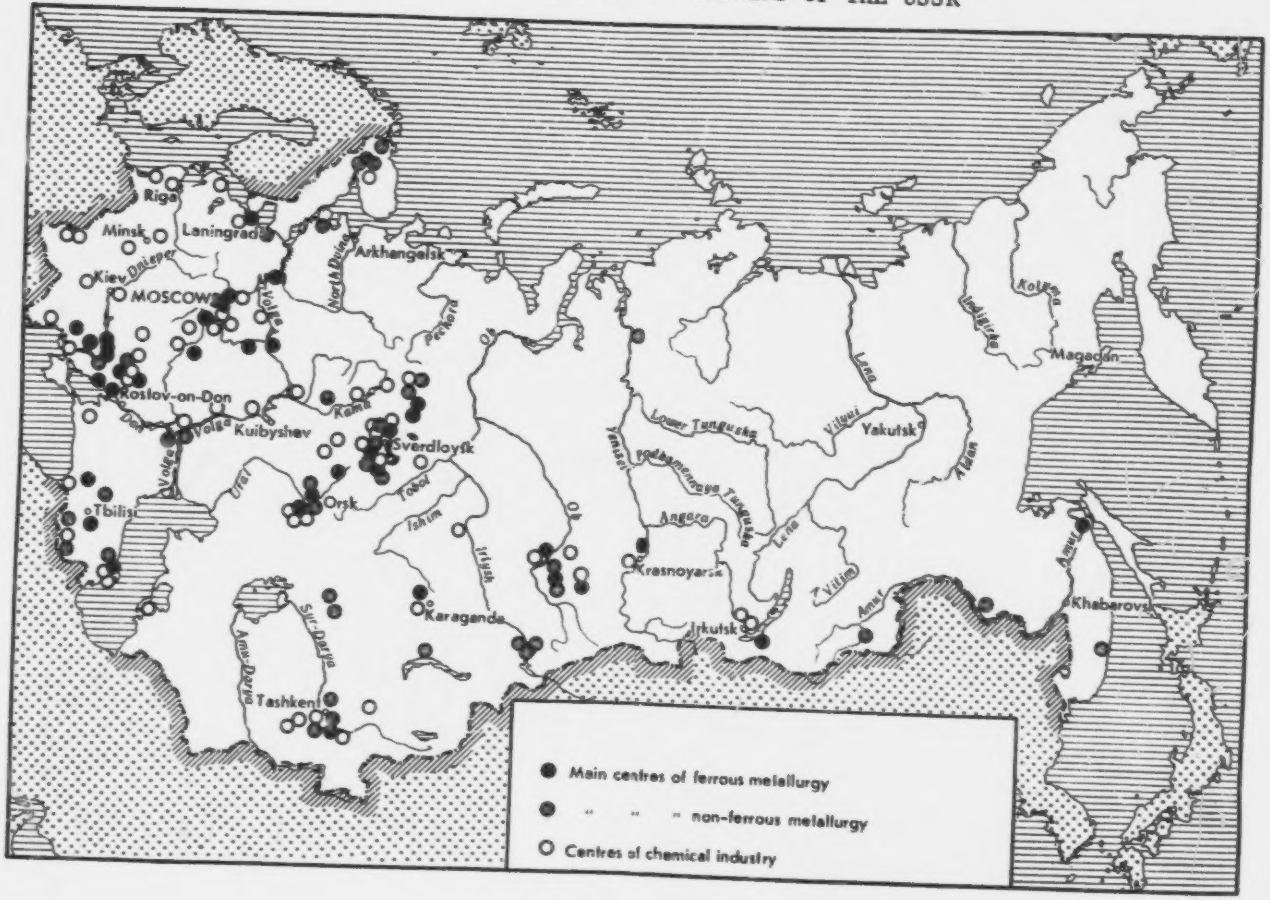
Large industrial systems are being created - Kustanai, Pavlodar-Ekibastuz, Achinsk-Krasnoyarsk, Bratsk-Taishet and a number of others. Ferrous and non-ferrous metallurgy, pulp and paper, hydrolysis and saw-milling industries are being established in the Bratsk-Taishet industrial system. The Achinsk-Krasnoyarsk industrial system is becoming one of the largest centers of aluminum and chemical industries, and production of ferrous metals, cellulose, paper, and oil products.

Construction of the third metallurgical base has been launched in Siberia, and a new base of ferrous metallurgy, using the enormous local iron and coal resources, has been created in Kazakhstan. A high-capacity power system is being organized in the same areas. Non-ferrous metallurgy is being further developed in Kazakhstan, Central Asia and in Transbaikal areas. The pulp and paper, as well as the timber, industries are being developed at a fast rate in the forest areas of Siberia and the Far East.

Ferrous metallurgy is also developing in the European part of the country by utilizing the enormous iron ore resources of the Kursk Magnetic Anomaly and the Ukrainian deposits. Large new production systems are under construction in the North-West, along the Volga, in the Northern Caucasus and the Ukraine.

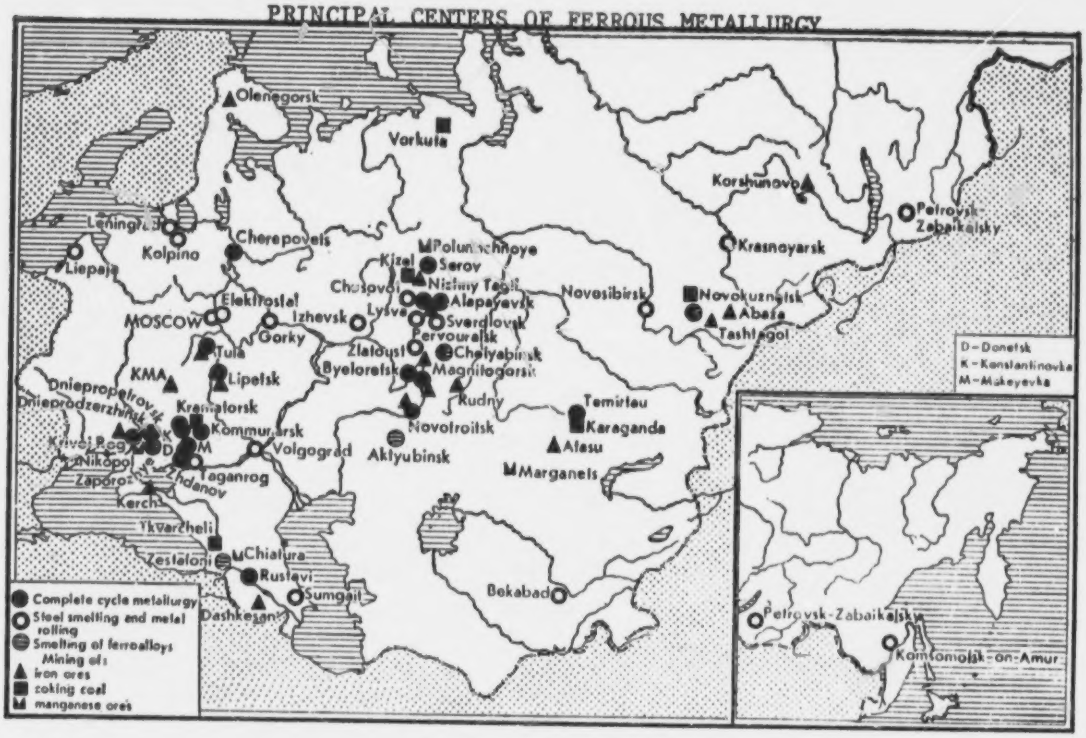
(After A. Lavrishchev, "Economic Geography
of the U.S.S.R.", Moscow 1969)

THE MAJOR INDUSTRIAL CENTERS OF THE USSR

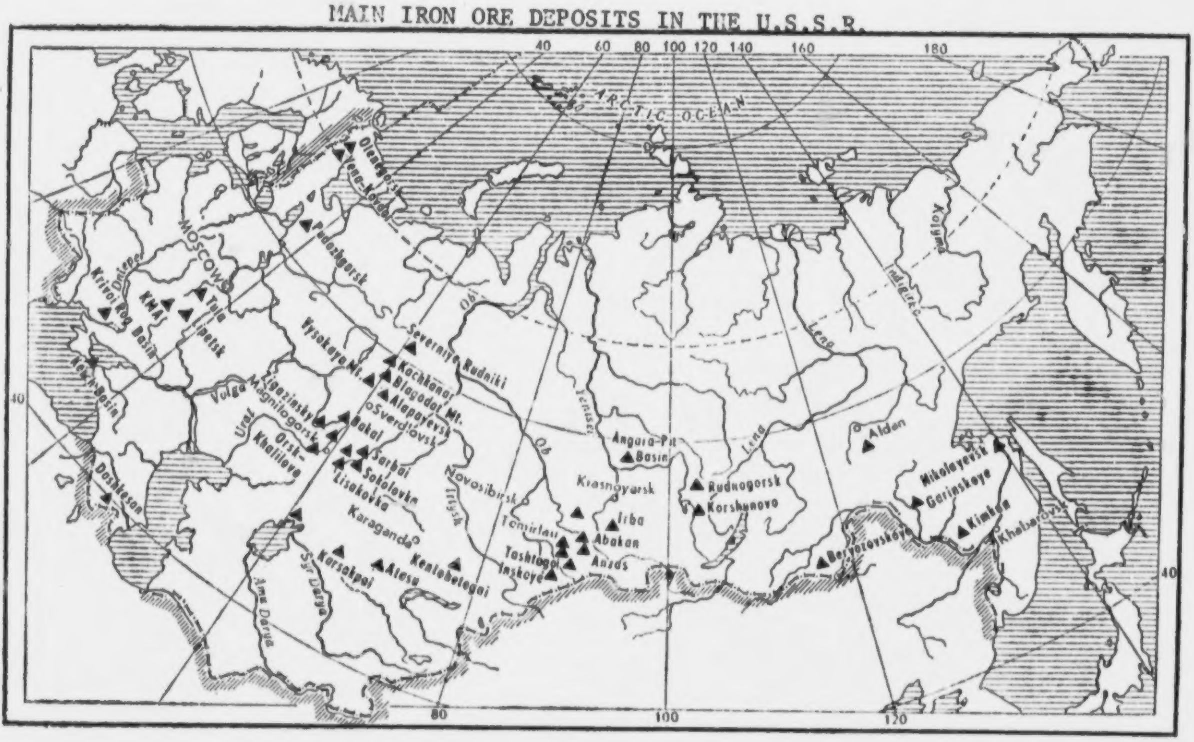


(After A. Efimov, "Soviet Industry", Moscow 1968)

PRINCIPAL CENTERS OF FERROUS METALLURGY

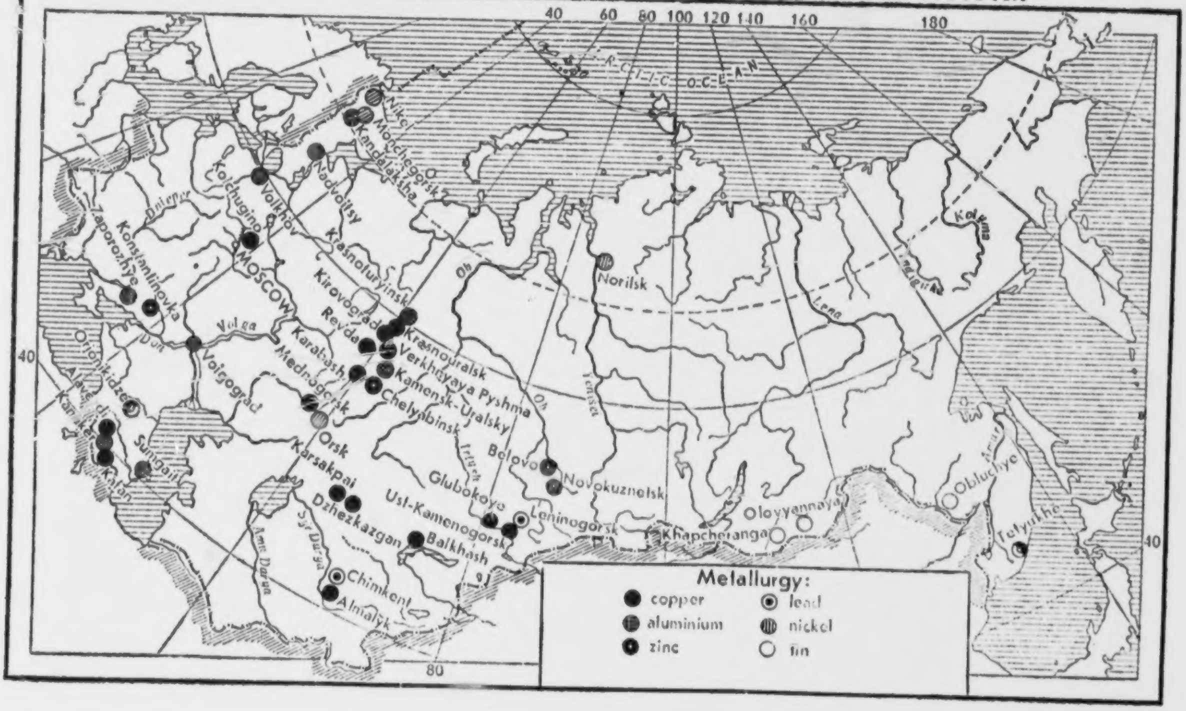


MAIN IRON ORE DEPOSITS IN THE U.S.S.R.

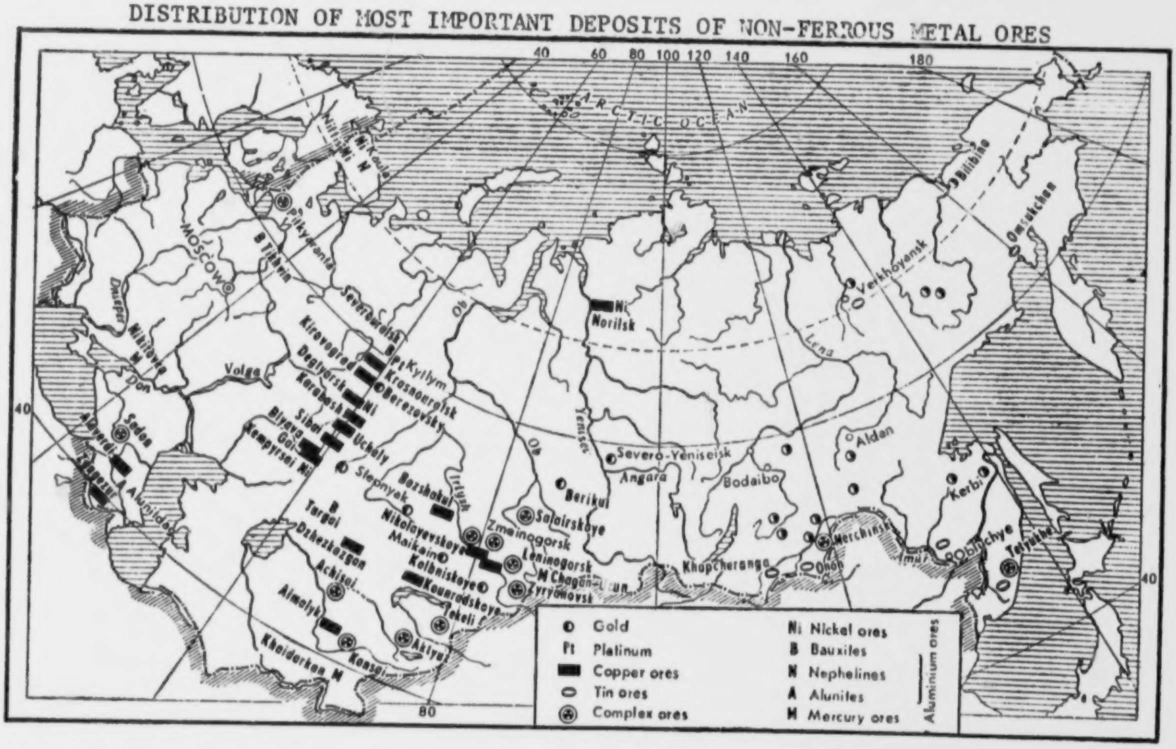


(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

PRINCIPAL CENTERS OF NON-FERROUS METALLURGY IN THE U.S.S.R.

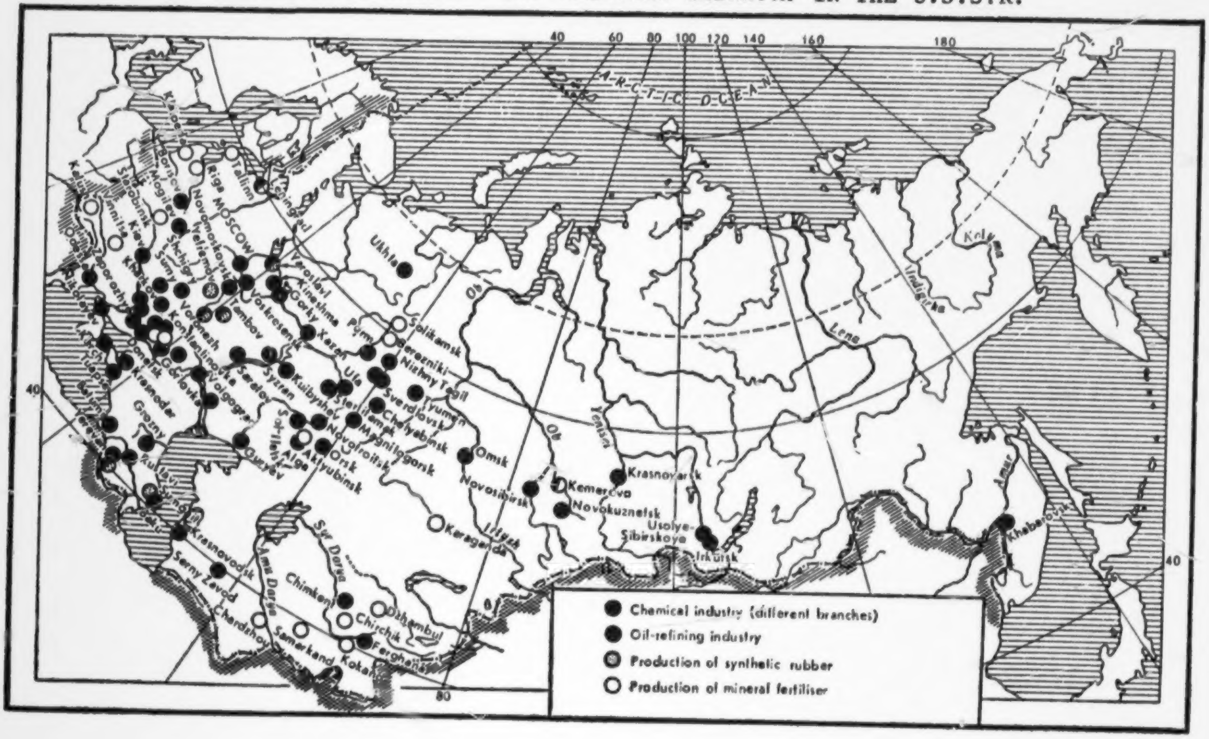


DISTRIBUTION OF MOST IMPORTANT DEPOSITS OF NON-FERROUS METAL ORES

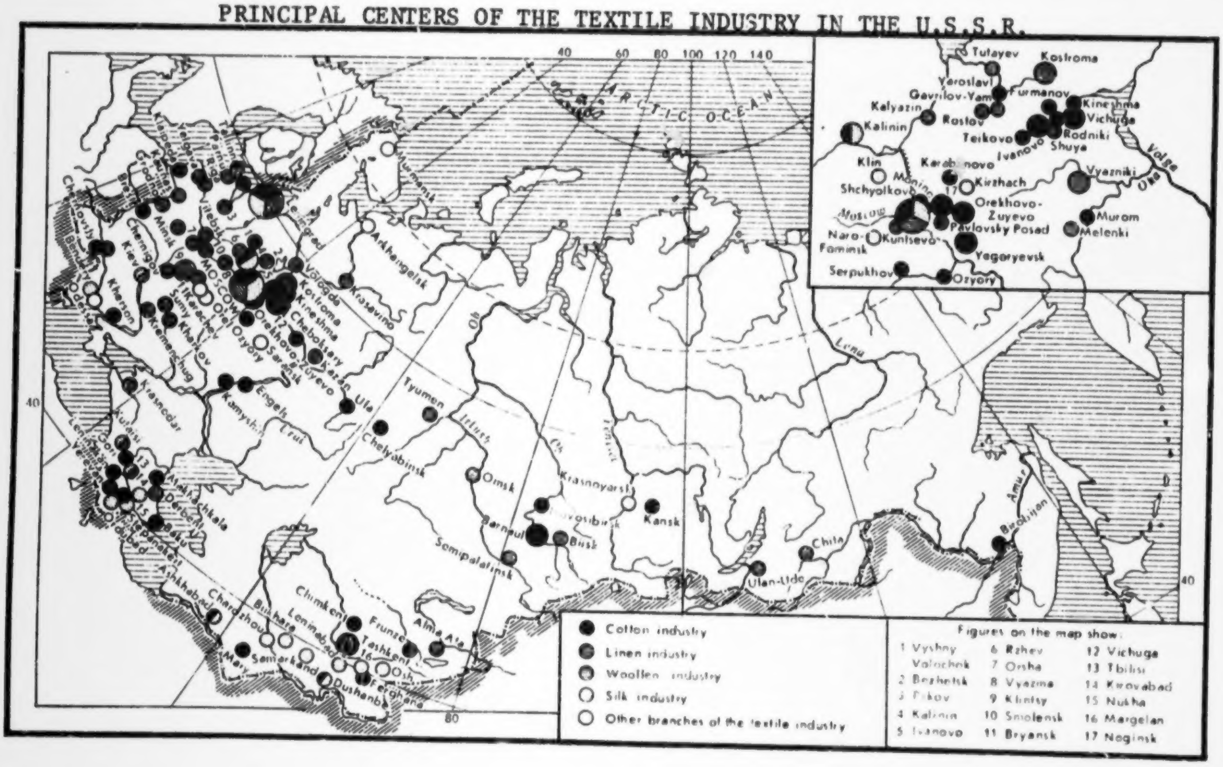


(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

PRINCIPAL CENTERS OF THE CHEMICAL INDUSTRY IN THE U.S.S.R.

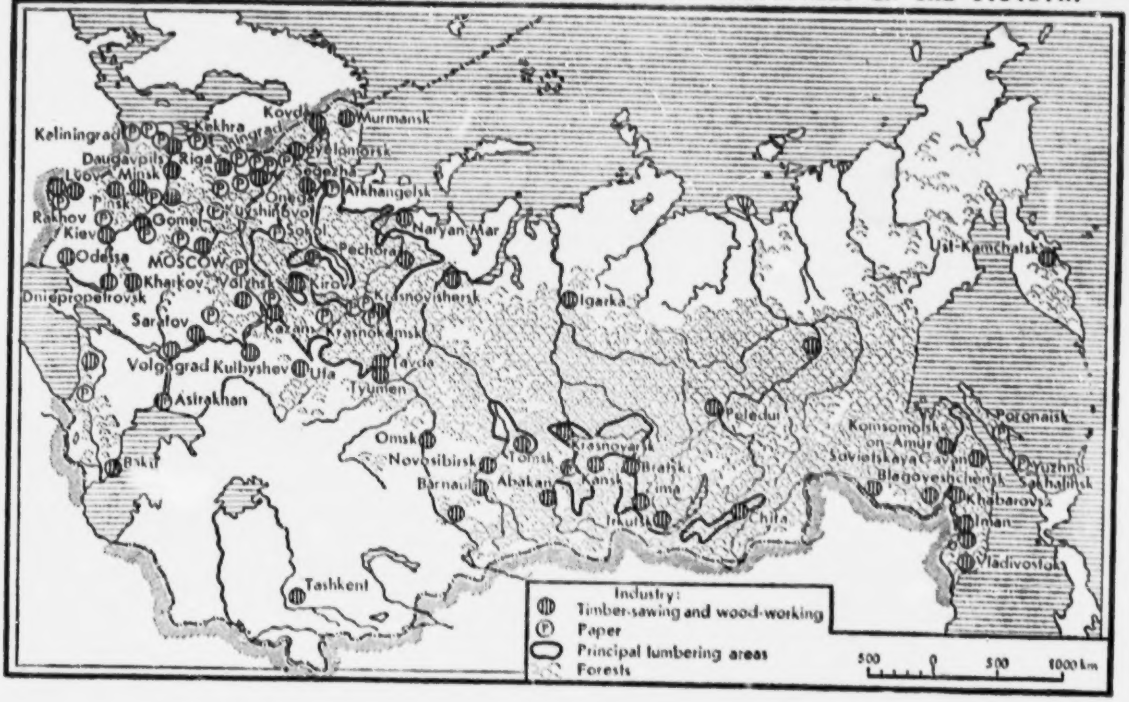


PRINCIPAL CENTERS OF THE TEXTILE INDUSTRY IN THE U.S.S.R.



(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

PRINCIPAL CENTERS OF WOOD-WORKING AND PAPER INDUSTRIES IN THE U.S.S.R.



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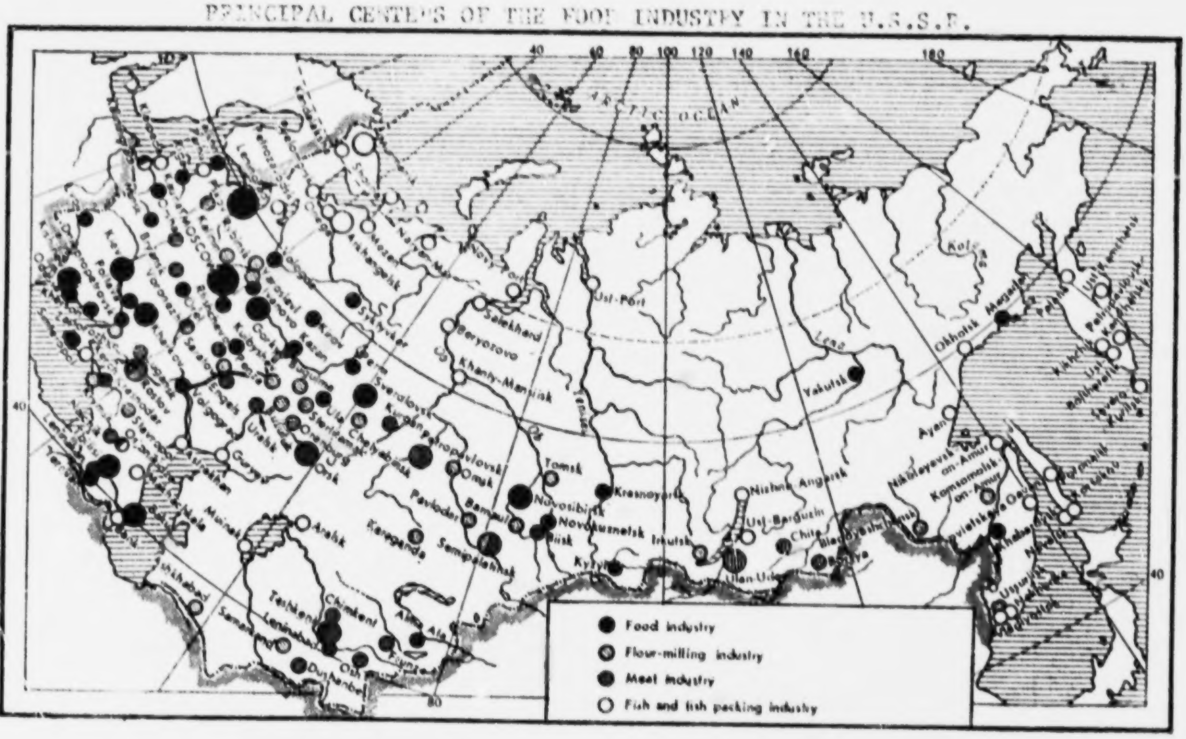
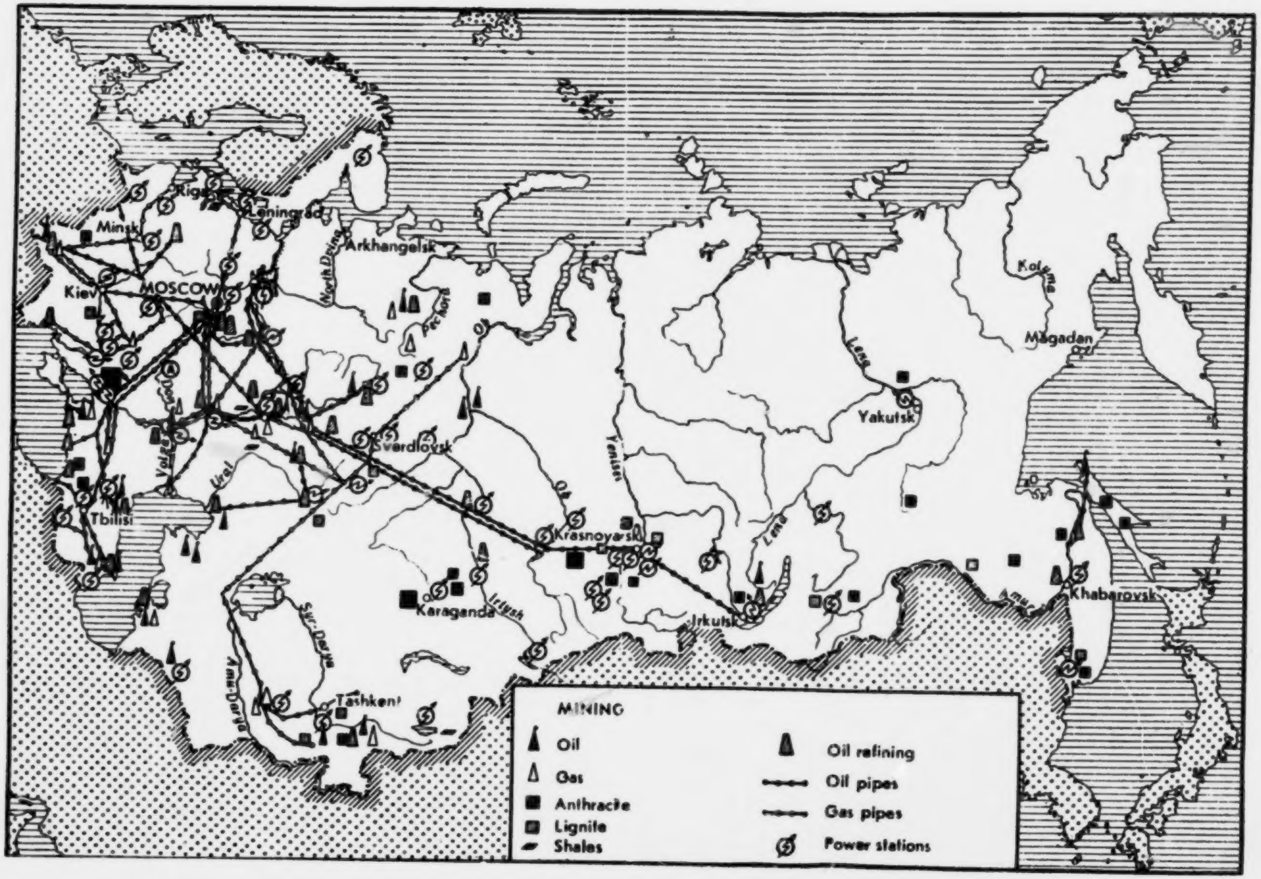


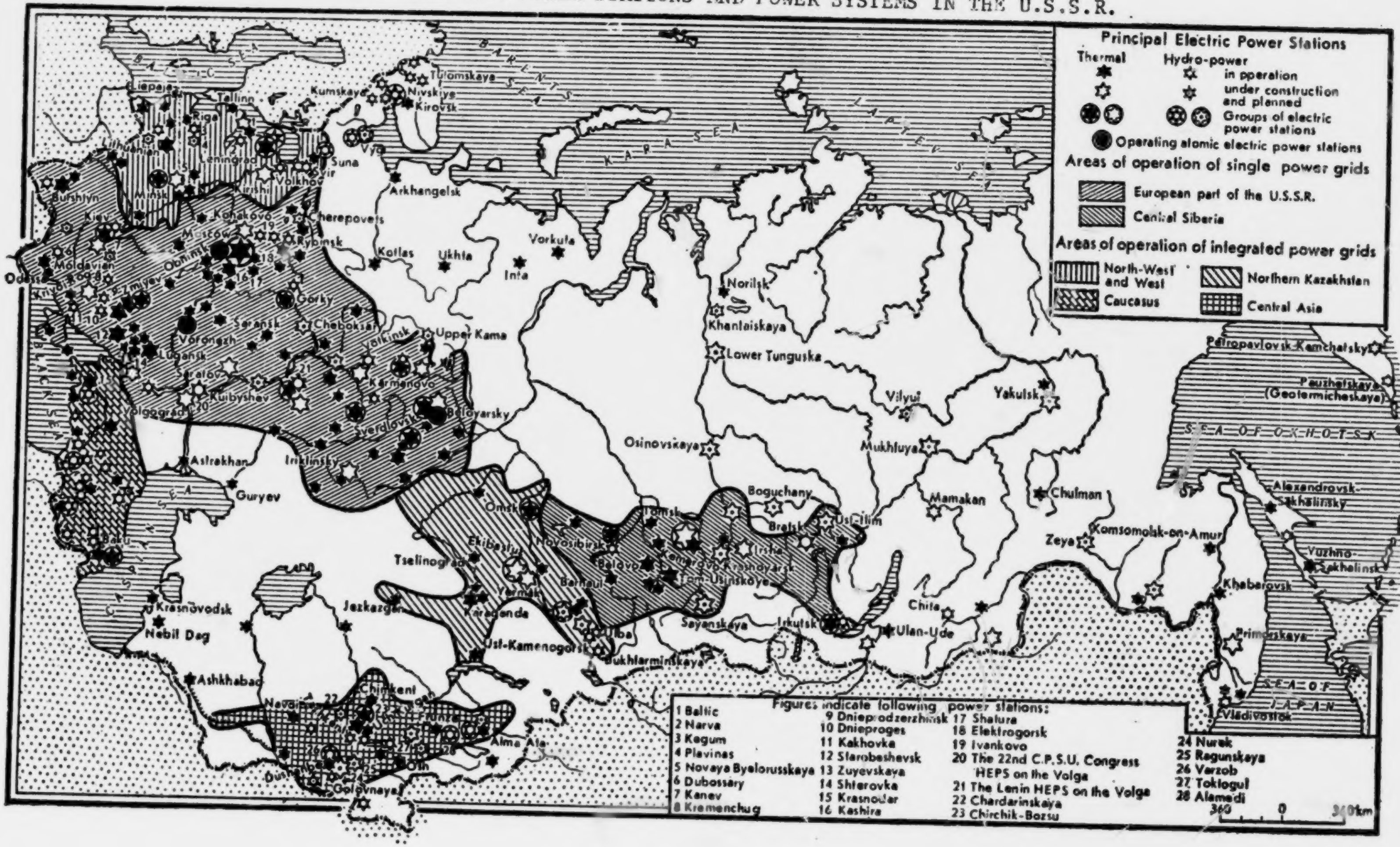
(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

THE MAIN MINING CENTERS OF THE USSR



(After A. Efimov, "Soviet Industry", Moscow 1968)

PRINCIPAL ELECTRIC POWER STATIONS AND POWER SYSTEMS IN THE U.S.S.R.



(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

MAXIMUM PERMISSIBLE CONCENTRATIONS OF NOXIOUS SUBSTANCES IN THE ATMOSPHERIC AIR OF POPULATED AREAS*

(V. A. Ryazanov)

From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 11, Izdatel'stvo "Meditsina" Moskva, p. 201-204, (1968).

Pollutant	Concentration, mg/m^3	
	Maximum single	Mean daily
1	2	3
1. Nitrogen dioxide	0,085	0,085
2. Nitric acid (based on HNO_3 molecule	0.4	0.4
(based on hydrogen ion)	0,006	0.006
3. Acrolein	0,30	0.10
4. Alpha-methylstyrene	0,04	0.04
5. Alpha-naphthoquinone	0,005	0.005
6. Amyl acetate	0,10	0,10
7. Amylene	1,5	1.5
8. Ammonia	0.20	0.20
9. Aniline	0,05	0.03
10. Acetaldehyde	0.01	——
11. Acetone	0,35	0.35
12. Acetophenone	0.003	0,003
13. Benzene	1.5	0,8
14. Gasoline (low-sulfur petroleum gasoline in terms of "C")	5,0	1,5
15. Shale gasoline (in terms of "C")	0,05	0.05
16. Butane	200.0	——
17. Butyl acetate	0.10	0,10
18. Butylene	3,0	3,0
19. Butyl alcohol	0.3	——
20. Butyl phosphate	0,01	——
21. Valeric acid	0.03	0,01
22. Vanadium pentoxide	——	0.002

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* Approved by the Assistant Chief Public Health Physician of the USSR on 12 September 1967, No. 692-67.

1	2	3
23. Vinyl acetate	0.20	0.20
24. Hexamethylenediamine	0.001	0.001
25. Bivinyl	3.0	1.0
26. Diketene	0.007	—
27. Dimethylaniline	0.0055	—
28. Dimethyl sulfide	0.08	—
29. Dimethyl disulfide	0.7	—
30. Dimethylformamide	0,03	0.03
31. Dowtherm	0.01	0.01
32. Dichloroethane	3.0	1.0
33. 2,3-Dichloro-1,4-naphthoquinone	0.05	0.05
34. Diethylamine	0.05	0.05
35. Isopropylbenzene	0.014	0.014
36. Isopropylbenzene hydroperoxide	0.007	0.007
37. Caprolactam (vapors, aerosol)	0.06	0.06
38. Caproic acid	0.01	0.005
39. Malathion	0.015	—
40. Xylene	0.2	0.2
41. Maleic anhydride (vapors, aerosol)	0,2	0.05
42. Manganese and its compounds (in terms of MnO_2)	—	0.01
43. Butyric acid	0.015	0.01
44. Mesidine	0.003	—
45. Methanol	1.0	0.5
46. Metaphos	0.008	—
47. Metachlorophenyl isocyanate	0.005	0.005
48. Methyl acrylate	0.01	—
49. Methyl acetate	0.07	0.07
50. Methyl mercaptan	$9 \cdot 10^{-6}$	—
51. Methyl methracylate	0.1	0.1
52. Monomethylaniline	0.04	—
53. Arsenic (inorganic compounds other than arsine, in terms of AS)	—	0.003
54. Nitrobenzene	0.008	0.008
55. Parachloroaniline	0.04	—
56. Parachlorophenyl isocyanate	0.0015	0.0015
57. Pentane	100.0	25.0
58. Pyridine	0.08	0.08
59. Propylene	3.0	3.0
60. Propyl alcohol	0.3	—
61. Nontoxic dust	0.5	0.15
62. Metallic mercury	—	0.0003

1	2	3
63. Soot (carbon black)	0.15	0.05
64. Lead and its compounds (other than tetraethyl lead) in terms of Pb	——	0,0007
65. Lead sulfide	——	0.0017
66. Sulfuric acid (based on H_2SO_4 molecule)	0.3	0.3
(based on hydrogen ion)	0.006	0.006
67. Sulfur dioxide	0.5	0,05
68. Hydrogen sulfide	0.008	0.008
69. Carbon disulfide	0.03	0.01
70. Hydrochloric acid (based on HCl molecule)	0.2	0.2
(based on hydrogen ion)	0,006	0.006
71. Styrene	0.003	0,003
72. Thiophene	0.6	——
73. Toluylene diisocyanate	0.05	0.02
74. Toluene	0.6	0.6
75. Trichloroethylene	4.0	1.0
76. Carbon monoxide	3.0	1.0
77. Acetic acid	0.2	——
78. Acetic anhydride	0.1	——
79. Phenol	0.01	0.01
80. Formaldehyde	0.035	0.012
81. Phosphoric anhydride	0.15	0.05
82. Phthalic anhydride (vapors, aerosol)	0.10	
83. Fluorine compounds (in terms F)		
Gaseous compounds (HF, SiF_4)	0.02	0.005
Soluble inorganic fluorides (NaF, Na_2SiF_6)	0.03	0.01
Sparingly soluble inorganic fluorides (AlF_3, Na_3AlF_3, CaF_2)	0.2	0,03
In the combined presence of gaseous fluorine and fluorine salts	0.03	0,01
84. Furfural	0.05	0.05
85. Chlorine	0.10	0,03
86. Chlorobenzene	0.10	0.10
87. Chloropropene	0.10	0.10
88. Hexavalent chromium (in terms of CrO_3)	0.0015	0.0015
89. Cyclohexanol	0.06	0.06
90. Cyclohexanone	0.04	0.04
91. Carbon tetrachloride	4.0	——
92. Epichlorhydrin	0.2	0,2
93. Ethanol	5.0	5.0
94. Ethyl acetate	0.1	0.1

1	2	3
95. Ethylene	3.0	3.0
96. Ethylene oxide	0.3	0.03

REMARKS

1. In the combined presence in atmospheric air of several substances possessing a summation effect, the sum of their concentrations as calculated by the formula below (§ 2) should not exceed 1 for:

a) acetone and phenol
b) sulfur dioxide and phenol
c) sulfur dioxide and nitrogen dioxide
d) sulfur dioxide and hydrogen fluoride
e) sulfur dioxide and sulfuric acid aerosol
f) hydrogen sulfide and dowtherm
g) isopropylbenzene and isopropylbenzene hydroperoxide
h) furfural, methanol and ethanol
i) strong mineral acids (sulfuric, hydrochloric and nitric) in terms of the hydrogen ion concentration (H)
j) ethylene, propylene, butylene and amylene

should not exceed 1.3 for:

a) acetic acid and acetic anhydride

should not exceed 1.5 for:

a) acetone and acetophenone
b) benzene and acetophenone
c) phenol and acetophenone

2. Formula for the calculation:

$$X = \frac{a}{m_1} + \frac{b}{m_2} + \frac{c}{m_3} \ldots,$$

where X is the unknown total concentration:

$\frac{a}{m_1} + \frac{b}{m_2} + \frac{c}{m_3}$ — is the concentration of the substance being determined, divided by the corresponding maximum permissible concentration for isolated action.

3. In the combined presence in atmospheric air of:

a) hydrogen sulfide and carbon disulfide
b) carbon monoxide and sulfur dioxide
c) phthalic and maleic anhydrides and alpha-naphthoquinone, the maximum permissible concentrations for each of them individually are retained.

4. In the combined presence in atmospheric air of parachlorophenyl isocyanate and metachlorophenyl isocyanate, temporarily, until a method of their isolated determination is developed, the standardization should be made on the more toxic substance, i. e., parachlorophenyl isocyanate.

5. The maximum permissible concentrations of noxious substances in the atmospheric air of populated areas as formulated in December 1966 (No. 655-66), should be considered obsolete.

DATA FOR THE SANITARY VALIDATION OF MAXIMUM PERMISSIBLE CONCENTRATIONS OF SULFUR DIOXIDE IN COMBINATION WITH PHENOL

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Among the many sources of atmospheric pollution, ferrous metallurgical plants are particularly important. At the present time, for economic reasons, by-product coke plants are built near metallurgical ones, since a modern metallurgical complex requires large amounts of coking coal for its production.

By-product coke plants discharge the most varied chemical substances into the atmosphere: sulfur dioxide, phenols, hydrogen sulfide, naphthalene, cyanide compounds, benzene, hydrocarbons, dust, soot, and other compounds, which can spread over considerable distances.

Ye. V. Yelfimova (1958), in a study of atmospheric pollution in the zones around the Magnitogorsk metallurgical complex over distances up to 7000 m, observed that the highest single concentrations of sulfur dioxide are 25 times the maximum permissible value.

According to the data of Yu. V. Neyman and V. V. Smolin (1964), as a result of the setting in operation of a large sintering plant in the Magnitogorsk metallurgical complex, the mean annual sulfur dioxide content in 1962 in a three-kilometer zone increased by a factor of 3, and at a distance of 7000 m, by a factor of 5, as compared with 1958.

The highest single concentrations of phenol in atmospheric air around the Novo-Lipetsk metallurgical plant, which includes a by-product coke plant, at a distance of 500-1500 m, exceeded the maximum permissible value by a factor of 105-15 (data of the Lipetsk regional sanitary-epidemiological station for 1962).

According to the data of D. N. Kalyuzhnyy and M. P. Gordynya (1964), the phenol concentration at a distance of 1000 m from a ferrous metallurgical plant exceeds the maximum permissible value by a factor of 60, and at a distance of 3000 m, by a factor of 85.

In order to estimate the degree of pollution of atmospheric air with sulfur dioxide and phenols, we studied the pollution in zones around plants whose composition included coke-chemical sections.

Three metallurgical plants (Orsk-Khalilovo, Cherepovets, and Novo-Lipetsk) were chosen for the study. The survey was made according to seasons (spring, summer, autumn.

To determine sulfur dioxide, we used a colorimetric method developed by M. V. Alekseyeva and R. Ya. Samorodina (1963). To determine phenol in atmospheric air, the method proposed by V. A. Khrustaleva (1963) was employed. The samples were analyzed on a photoelectrocolorimeter (FEK-M).

The results of our studies are presented in Tables 1 and 2.

Table 1

Highest Single Sulfur Dioxide Concentrations in the Zones Around Three Metallurgical Plants.

Distance From Source of Discharge, m	Highest Single Sulfur Dioxide Concentrations, mg/m^3		
	Plant No. 1[1]	Plant No. 2[2]	Plant No. 3[3]
500	3,4	0,88	0,80
1 000	4,8	0,70	0,45
3 000	6,5	0,09	2,6
5 000	1,6	0,06	0,4
7 000	0,68	0,02	0,1
10 000	0,3	—	—

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[1] Orsk-Khalilovo metallurgical complex

[2] Cherepovets metallurgical plant

[3] Novo-Lipetsk metallurgical plant

Table 2

Highest Single Phenol Concentrations in the Zones Around Three Metallurgical Plants.

Distance From Source of Discharge, m	Highest Single Phenol Concentrations, mg/m^3		
	Plant No.1	Plant No. 2	Plant No. 3
500	4,5	0,38	0,18
1 000	5,0	0,28	0,35
3 000	2,58	0,13	0,18
5 000	0,58	0	0,035
7 000	0,178	0	0
10 000	0,025	0	0

The highest sulfur dioxide concentrations around the first metallurgical plant were determined in the residential zone at a distance of 3-5 km from the source of discharge. The concentrations found exceeded the maximum permissible level by a factor of 13-3.2. A slight excess over the maximum permissible concentration was noted even at a distance of 7000 m from the complex.

In addition to the by-product coke plant, a major source of pollution of atmospheric air with sulfur dioxide was the sintering plant, operating on high-sulfur stock (up to 5% sulfur) and discharging process gases without preliminary purification through a stack 60 m high.

The heaviest pollution with phenol was observed at a distance of up to

3000 m from the sources of discharge. Thus, in the 3000 m zone, the highest single concentration was 2.58 mg/m^3. Beyond that distance, the pollution level dropped markedly, but an excess over the maximum permissible concentration was also observed in more distant zones.

During the collection of samples at a distance of up to 3000 m from the complex, all the persons collecting the samples complained of an unpleasant odor and an irritating effect of the gases on the upper respiratory tract.

The sulfur dioxide concentrations at a distance of 500-1000 m from the second metallurgical plant exceeded the maximum permissible value by a factor of 1.7-1.4. In all the succeeding zones (3000, 5000 and 7000m), the pollution levels were found to be considerably below the maximum permissible concentration.

Pollution with phenol at a distance of 500 m from the points of discharge exceeded the maximum permissible value by a factor of 30-38. At a distance of 1000 m, the phenol concentrations decreased, but they surpassed the maximum permissible value by a factor of 13-10; phenol could not be detected at a distance of 5000 m from the sources of discharge.

Data of the study around the third metallurgical plant showed that the highest sulfur dioxide concentrations were observed in the 3000 m zone (residential area), where the highest single maximum permissible concentration was exceeded by a factor of 5.2-3.8. The highest single concentrations at a distance of 500 m surpassed the maximum permissible value by a factor of 1.6. In all the remaining zones (1000, 5000 and 7000 m), the sulfur dioxide content of the samples was substantially below the level of the maximum permissible concentration.

The phenol concentrations according to zones around this metallurgical plant at a distance of 500 m exceeded the maximum permissible level by a factor of 18-10, in the 1000 m zone by a factor of 35-20, 3000 m by a factor of 18-7, and at a distance of 5000 m, by a factor of 3½-2. No phenol could be detected in more distant zones.

The heavy pollution of atmospheric air observed around the Orsk-Khalilovo metallurgical complex served as the basis for the study of the influence of these discharges on the health of children living in the area of the industrial facility in question. To this end, in collaboration with a group of clinicians, we made an extensive medical survey of the school children living in two neighborhoods, one of which was used as the control. The total number of school children surveyed in the groups was 381-387, and the age-sex composition, social and material conditions and length of residence (no less than 5 years) in the two neighborhoods were approximately the same.

The results of the survey showed that in children who lived in the gas-polluted area there was a significant lag in physical development from the standpoint of growth, weight, circumference of chest, and muscular strength. The children complained twice as often of headache, dizziness, nausea, and epigastric pain. They also complained of an increased fatigue and excitability. As compared with the control group, the schoolchildren of the gas-polluted area had five times as much chronic inflammatory afflictions of the larynx and three times as much tonsillitis, adenoiditis, and atrophic and hypertrophic rhinopharyngitis. Treatment of data on first complaints showed that the disease rate of the upper respiratory tract in schoolchildren of the gas-polluted area was three times higher than in the control. Biochemical studies detected from 0.005 to 0.12 mg% sulfur dioxide in schoolchildren of the polluted area, indicating a heavy and steady air pollution, since no sulfur dioxide is found in the blood under normal conditions. The presence of sulfur dioxide in the children's blood shows an adverse influence on the defensive functions of the organism, as confirmed by the amount of vitamin C in the blood and the extent of its excretion from the body with the urine.

In order to explain the influence of discharges of the metallurgical complex on the sanitary living conditions and health of the city's inhabitants, the population living at various distances from the third metallurgical plant was interrogated. A total of 372 persons were questioned. Eighty-two percent of the persons interrogated, living at distances of up to 5000 m on the leeward side of the source of discharges, noted that the metallurgical plant polluted the air with foul-smelling gases that caused irritation the year around. The population living at a distance of 7000 m from the plant had fewer such complaints. However, they did point out the destruction of green plantings and vegetable crops.

According to literature data, the toxic effect of sulfur dioxide and phenol was chiefly studied in large concentrations (N. V. Lazarev, I. V. Sidorenko, L. S. Rozanov, E. E. Grigor'yev, Greenwald, Deichmann, Kitzmiller, and others).

The biological effect of low concentrations of sulfur dioxide and phenol was studied by F. I. Dubrovskaya (1957), B. M. Mukhitov (1963), and K. A. Bushtuyeva (1964).

According to Dubrovskaya's data (1957), the threshold of smell for sulfur dioxide has been established at a level of 1.6-2 mg/m^3, and according to Bushtuyeva's data (1964), at 1.5-1.8 mg/m^3; the threshold of the effect on the light sensitivity of a dark-adapted eye is around 0.6 mg/m^3 (Dubrovskaya, 1957) and 0.9 mg/m^3 (Bushtuyeva, 1964). The threshold of the reflex effect on the electrical activity of the brain, obtained by the method of depression of the alpha rhythm, is 0.9-0.85 mg/m^3, and by the method of the electrocortical conditioned reflex, 0.6 mg/m^3 (Bushtuyeva, 1964).

According to Mukhitov's data (1963), the olfactory threshold of phenol was 0.022 mg/m^3. The minimum active concentration for the light sensitivity of the eye was found to be 0.0155 mg/m^3. The threshold of the reflex effect on the electrical activity of the brain lies at the same level.

Our objective was to study the biological effect of these substances when they are present together.

The odor threshold of sulfur dioxide was determined on 19 subjects (Table 3). Concentrations from 3.2 to 0.22 mg/m^3 were studied. As was shown by the results, 10 persons out of 19 perceived the odor of sulfur dioxide in a concentration of 0.88-0.87 mg/m^3, which is one-half the threshold of smell determined by F. I. Dubrovskaya (1957) and K. A. Bushtuyeva (1964).

The determination of the olfactory threshold of phenol was also carried out on the same 19 subjects (see Table 3). A concentration of 0.022 mg/m^3 was found to be the minimum perceptible value, and a concentration of 0.016 mg/m^3, the maximum imperceptible value, i. e., our data confirmed the results of Mukhitov (1963). The odor threshold was then determined on the same 19 subjects for the combined presence of sulfur dioxide and phenol (see Table 3). The olfactory threshold for a mixture of sulfur dioxide and phenol present together was 0.44 mg/m^3 for sulfur dioxide and 0.011 mg/m^3 for phenol in the most sensitive persons (five out of 19); it was 0.33 mg/m^3 (sulfur dioxide) and 0.011 mg/m^3 (phenol) for two subjects, and the concentrations of 0.22 mg/m^3 sulfur dioxide and 0.005 mg/m^3 phenol were imperceptible for them.

As is evident from Table 3, the odor of a mixture of sulfur dioxide and phenol is perceived for a total concentration index close to or equal to unity, indicating a simple summation of the effects.

Adaptometric observations were made on three subjects for whom the odor threshold was: I. V., 0.33 mg/m^3 (sulfur dioxide) and 0.011 mg/m^3 (phenol); K. S., 0.44 mg/m^3 (sulfur dioxide) and 0.011 mg/m^3 (phenol); N. T., 0.33 mg/m^3 (sulfur dioxide) and 0.011 mg/m^3 (phenol). Three combinations of the substances in question were studied. In all of the subjects, sulfur dioxide and phenol concentrations equal to 0.88-0.022 mg/m^3 and 0.44-0.011 mg/m^3 respectively caused a statistically significant change of the light sensitivity, while concentrations of 0.22 mg/m^3 sulfur dioxide and 0.005 mg/m^3 phenol were found to be inactive in statistical treatment.

We subsequently conducted a series of electroencephalographic tests using the technique of A. D. Semenenko (1963). They were carried out on four persons with the lowest olfactory threshold for sulfur dioxide and phenol present together. The potentials of the cerebral cortex were taken off by the bipolar method and recorded with a four-channel ink-recording encephalograph of domestic manufacture. The tests were carried out no fewer than three times with each mixture. The concentrations, taken in the proportion of one-half the thresholds of olfactory perception, 0.44 mg/m^3 (sulfur dioxide) and 0.011 mg/m^3 (phenol), caused a statistically significant change in the electrical activity of

Table 3

Thresholds of Olfactory Perception of Sulfur Dioxide and Phenol During Isolated and Combined Action

Subject	Sulfur Dioxide, mg/m^3		Phenol, mg/m^3		Mixture of Sulfur Dioxide and Phenol, mg/m^3			Threshold of Sulfur Dioxide for Combined Action	Threshold of Phenol For Combined Action	Total Concentration Expressed in Fractions of the Threshold Concentration of each Constituent	Proposed Maximum Permissible Concentration for Combined Action
					Smell Threshold		Subthreshold Concentration for Smell	Odor Threshold of Sulfur Dioxide for Isolated Action	Odor Threshold of Phenol for Isolated Action		Maximum Permissible Concentration for Isolated Action in Parts
	Smell Threshold	Subthreshold Concentration for Smell	Smell Threshold	Subthreshold Concentration for Smell	Sulfur Dioxide	Phenol					
B. Z.	1,75	1,62	0,078	0,064	0,88	0,038	0,64—0,026	$\frac{0,88}{1,75}=0,50$	$\frac{0,038}{0,078}=0,48$	0,98	
B. L.	1,75	1,62	0,078	0,064	0,88	0,038	0,64—0,026	$\frac{0,88}{1,75}=0,50$	$\frac{0,038}{0,078}=0,48$	0,98	
G. A.	0,87	0,65	0,023	0,016	0,44	0,011	0,36—0,005	$\frac{0,44}{0,87}=0,50$	$\frac{0,011}{0,023}=0,47$	0,97	
D. N.	1,75	1,62	0,094	0,078	0,88	0,047	0,64—0,026	$\frac{0,88}{1,75}=0,50$	$\frac{0,047}{0,094}=0,50$	1,0	
Ye. N.	2,4	2,25	0,094	0,078	1,2	0,047	1,0—0,039	$\frac{1,2}{2,4}=0,50$	$\frac{0,047}{0,094}=0,50$	1,0	
Ye.Ye.	0,88	0,65	0,032	0,23	0,44	0,016	0,36—0,011	$\frac{0,44}{0,88}=0,50$	$\frac{0,016}{0,032}=0,50$	1,0	
I. V.	0,87	0,65	0,023	0,016	0,33	0,01	0,22—0,005	$\frac{0,33}{0,87}=0,37$	$\frac{0,011}{0,023}=0,47$	0,84	$\frac{0,22}{0,50}=\frac{0,005}{0,01}=$ $=0,44+0,50=0,94$
K. Ye.	0,88	0,65	0,035	0,023	0,48	0,016	0,36—0,011	$\frac{0,48}{0,88}=0,54$	$\frac{0,016}{0,035}=0,46$	1,0	
K. S.	0,87	0,65	0,023	0,016	0,44	0,011	0,36—0,005	$\frac{0,44}{0,87}=0,50$	$\frac{0,011}{0,023}=0,47$	0,97	
K. T.	0,88	0,65	0,022	0,016	0,44	0,011	0,36—0,005	$\frac{0,44}{0,88}=0,50$	$\frac{0,011}{0,022}=0,50$	1,0	
L. G.	0,87	0,65	0,035	0,023	0,48	0,016	0,36—0,011	$\frac{0,48}{0,87}=0,55$	$\frac{0,016}{0,035}=0,45$	1,0	
L. V.	2,4	2,25	0,094	0,078	1,2	0,048	1,0—0,039	$\frac{1,2}{2,4}=0,50$	$\frac{0,048}{0,094}=0,50$	1,0	
M. A.	0,88	0,65	0,035	0,016	0,48	0,016	0,36—0,005	$\frac{0,48}{0,88}=0,54$	$\frac{0,016}{0,035}=0,46$	1,0	
N. T.	0,88	0,65	0,022	0,016	0,33	0,011	0,22—0,005	$\frac{0,33}{0,88}=0,37$	$\frac{0,011}{0,022}=0,50$	0,87	
O. V.	2,4	2,25	0,094	0,078	1,2	0,048	1,0—0,039	$\frac{1,2}{2,4}=0,50$	$\frac{0,048}{1,094}=0,50$	1,0	
P. T.	2,4	2,25	0,094	0,078	1,2	0,048	1,0—0,039	$\frac{1,2}{2,4}=0,50$	$\frac{0,048}{0,094}=0,50$	1,0	
S. V.	0,88	0,65	0,023	0,016	0,44	0,011	0,36—0,005	$\frac{0,44}{0,88}=0,50$	$\frac{0,011}{0,023}=0,47$	0,97	
S. Ye.	1,75	1,62	0,078	0,064	0,88	0,038	0,64—0,026	$\frac{0,88}{1,75}=0,50$	$\frac{0,038}{0,078}=0,48$	0,98	
Sh. A.	1,75	1,62	0,078	0,064	0,88	0,038	0,64—0,026	$\frac{0,88}{1,75}=0,50$	$\frac{0,038}{0,078}=0,48$	0,98	

the cerebral cortex in all four subjects. Combined concentrations of 0.22 mg/m^3 (sulfur dioxide) and 0.005 mg/m^3 (phenol) were found to be inactive.

Fig. 1 shows the influence of inhalation of sulfur dioxide and phenol on the activity of the cerebral cortex in subject I. V.

Considering that in our tests the odor threshold concentrations for the most sensitive persons agreed with earlier established values for phenol only, and that we did not determine the thresholds of isolated action of sulfur dioxide and phenol with the aid of adaptometry and encephalography, the effect of the combined action of the substances under consideration was derived from the odor threshold as the most sensitive test. Calculations showed that the effect is close to a simple summation:

$\frac{0.33}{0.87}$ sulfur dioxide + $\frac{0.011}{0.022}$ phenol = 0.87 (see Table 3).

In order to refine the inactive concentrations of sulfur dioxide and phenol for their combined presence, we studied subthreshold concentrations (0.22 mg/m^3 - sulfur dioxide + 0.005 mg/m^3 - phenol), whose fractions of their highest single maximum permissible valurs added up to less than 1. In all of the techniques used, these proportions did not cause any statistically significant changes in the physiological reactions of the body. For this reason, as the highest single maximum permissible concentration of the sulfur dioxide - phenol mixture we recommend a proportion of the concentrations of these substances such that some of the fractions of their individual highest single maximum permissible levels does not exceed unity.

In order to detect the minimum functional shifts in the organism as a result of a prolonged action of a combination of the substances studied, we carried out a three-month round-the-clock dynamic exposure of white rats. Sixty white female rats divided into four equal groups were selected.

Rats of the first group were subjected to the action of 1.43 ± 0.02012 mg/m^3 sulfur dioxide and 0.49 ± 0.00488 mg/m^3 phenol, those of the second group to 0.45 ± 0.011168 mg/m^3 sulfur dioxide and 0.0498 ± 0.00112 mg/m^3 phenol, and those of the third group, to 0.046 ± 0.000928 mg/m^3 sulfur dioxide and 0.0051 ± 0.000058 mg/m^3 phenol; the fourth group was the control.

In our study, we used a series of tests characterizing the different aspects of the vital activity of the organism. Observations were made on the general condition of the animals, their weight, the latent reflex time by the chronoreflexometric method, coproporphyrin metabolism, cholinesterase activity, resistance of erythrocytes, hemoglobin index, fluorescent* leucocytes, and nucleic acids. At the end of the exposure, pathomorphological analyses of the internal organs were performed.

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* Editor's note: For the Russian terms "luminescent" and "luminescence" in this paper, we have substituted "fluorescent" and "fluorescence" on the basis of the definitions of these terms.

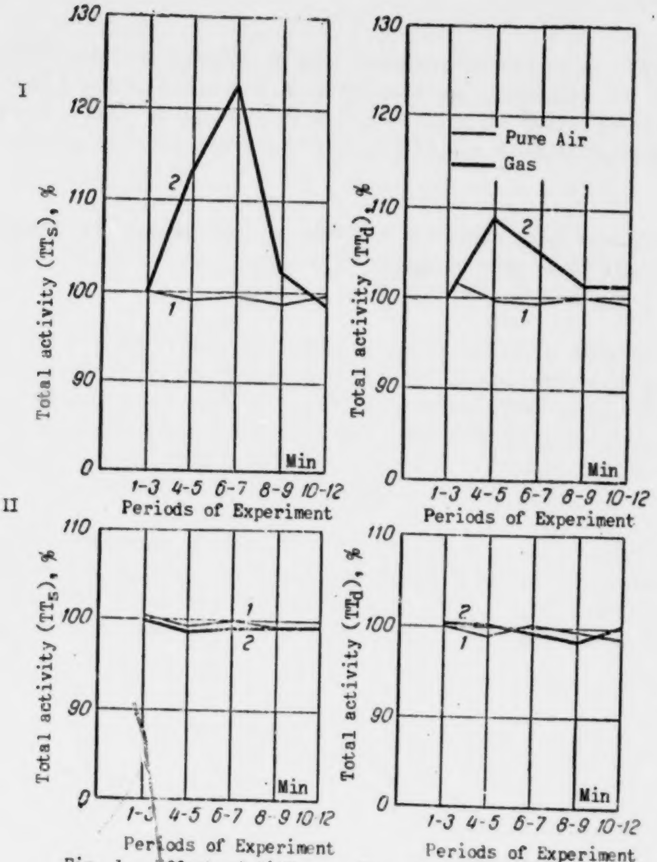

Fig. 1. Effect of different SO_2 and phenol concentrations present together on the electrical activity of the cerebral cortex in subject I. V.
I - sulfur dioxide concentration 0.44 mg/m^3, phenol concentration 0.011 mg/m^3; II - sulfur dioxide concentration 0.22 mg/m^3, phenol concentration 0.005 mg/m^3.
1 - pure air; 2 - gas.

In the course of the entire period of exposure, no substantial changes were observed in the general condition of the animals or their behavior. In all the chambers, the animals were equally active, ate well, and gained weitht regularly. No reliable weight changes were found in any experimental animals as compared with the controls.

In our studies, one of the sensitive tests was found to be the index of latent reflex time. The latent time was measured by means of a reflexometer of S. I. Gorshkov's design (1963). During the period of exposure, the studies were made once every ten days. The latent reflex time was estimated from the speed of the onset of the motor response. As a result of the toxic action of the substances which we studied, we observed a statistically significant shortening of the latent reflex time in the first and second groups, this being an indication of the predominance of excitation processes in the central nervous system.

In order to evaluate the chronic effect of low concentrations of sulfur dioxide and phenol when they are present together, we studied the porphyrin metabolism (M. I. Gusev and Yu. K. Smirnov, 1960).

The results of these studies showed that statistically significant shifts toward a decrease in the excretion of coproporphyrins with the urine were obtained in rats of the first and second groups on the 20th day of exposure. After ten days of the recovery period, the porphyrin metabolism in rats of these groups returned to normal. In rats of the third and fourth groups, no appreciable change in the porphyrin metabolism was observed.

A study of the cholinesterase activity showed a depression of the activity of this enzyme in rats of the first and second groups as compared with the control and the third group, indicating a disturbance of the compensatory functions of the body because of an intoxication of the nervous system.

Table 4

Combined Effect of Sulfur Dioxide and Phenol on the Organism of White Rats in the Course of Dynamic Exposure

Test Studied	Concentration, mg/m^3		
	Sulfur Dioxide 1.43 ± 0.02012, phenol 0.49 ± 0.00488	Sulfur Dioxide 0.45 ± 0.011168, phenol 0.0498 ± 0.00122	Sulfur Dioxide 0.046 ± 0.000928, phenol 0.0051 ± 0.000058
Weight and behavior	—	—	—
Chronoreflexometry	+	+	—
Coproporphyrin metabolism	+	+	—
Cholinesterase activity	+	+	—
Erythrocyte resistance	—	—	—
Hemoglobin index	—	—	—
Fluorescent leucocytes	+	+	—
Nucleic acids	—	—	—

+ significant changes; - insignificant changes

A study of a series of hematological indices showed a statistically significant increase of leucocytes with orange fluorescence. As a result of the toxic effect of sulfur dioxide and phenol on the physicochemical metabolism of a leucocyte cell, an orange fluorescence of leucocytes was observed in rats of the first and second groups under conditions of fluorescent microscopy. A particularly appreciable increase of the number of cells with an altered fluorescence was noted during the first period of exposure, and later it gradually decreased. No changes could be detected in the other indicators of the blood (erythrocyte resistance, hemoglobin). Nucleic acids did not give any statistically significant shifts either.

In a histopathological study of the internal organs and central nervous system, the rats of the first group showed slight signs of interstitial pneumonia in the lungs, degenerative changes in the kidneys, and a decrease of the glycogen function in the liver. Primary irritations of the large neurons of the cortex and of the brain stem were observed in the central nervous system. In rats of the second group, the above-indicated changes were not constant and rather slight, and they could not be detected in the third and fourth groups.

Thus, the results of the chronic experiment performed make it possible to recommend the following mean daily concentraion of the mixture: 0.05 mg/m^3 sulfur dioxide and 0.005 mg/m^3 phenol, which failed to produce statistically significant shifts in any of the tests.

Conclusions

1. The threshold of olfactory perception of sulfur dioxide in the most sensitive persons is 0.87 mg/m^3, and that of phenol, 0.022 mg/m^3. For combined presence, it is 0.33 mg/m^3 (sulfur dioxide) and 0.011 mg/m^3 (phenol). The effect of combined action is close to a simple summation.

2. When sulfur dioxide and phenol are jointly present in atmospheric air, their total highest single concentration, expressed in fractions of the maximum permissible values for each of them in isolated action, should not exceed unity.

3. The mean daily maximum permissible concentraion of sulfur dioxide and phenol for joint presence in atmospheric air is recommended at the following levels: sulfur dioxide 0.05 mg/m^3, and phenol, 0.005 mg/m^3.

4. Results of studies of atmospheric air around ferrous metallurgical plants including by-product coke departments show that the highest concentrations are found at distances of up to 3000 m from the source of discharge; beyond this distance, the pollution level drops off sharply.

LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1968 bibliography.

DATA FOR A SANITARY BASIS OF THE MEAN DAILY MAXIMUM PERMISSIBLE CONCENTRATION OF NITROGEN DIOXIDE IN ATMOSPHERIC AIR

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 11, Izdatel'stvo "Meditsina" Moskva, p. 164-171, (1968).

In atmospheric air, we find mainly NO_2. For this reason, of all the nitrogen oxides that are important in a sanitary sense, NO_2 stands apart from the others as a major air-pollutant.

Henderson and Haggard have also noted that whatever the molecular form in which NO_2 is inhaled, it undergoes changes corresponding to the temperature of the body. At a temperature of 40°C, approximately 30% of nitrogen dioxide is in the form of NO_2, and 70% in the form of N_2O_2. Both forms of nitrogen dioxide form nitric and nitrous acids by reacting with water.

In the respiratory tract, these acids combine with the alkalis of the tissues to form nitrates and nitrites, which have an irritating and caustic effect on the mucous membranes, particularly those of the deep areas of the respiratory tract, and a vasodilating effect with the formation of methemoglobin. In heavy poisoning with nitrogen oxides, toxic edema of the lungs may develop.

Nitrogen oxides are discharged into the atmosphere as a result of various industrial processes:

1) in the production of nitric acid and sulfuric acid (Mills-Packard process);
2) in the production of aniline dyes and explosives;
3) in metal etching, metal engraving, and autogenous welding;
4) in the production of rayon and mineral fertilizers, i. e., in processes involving nitration.

Nitrogen oxides are also present in gunpowder gases, exhaust gases of internal combustion engines, jet engines, and during the combustion of motion picture and x-ray film. Nitrogen oxides are evolved during the ensilage of grain (cited by N. V. Lazarev), during the operation of powerful gamma emitters, and in many other processes.

The literature contains publications dealing with experimental studies of the effect of relatively large concentrations of nitrogen oxides. Most of the studies were carried out under acute experimental conditions. There are virtually no experimental data on chronic exposure to low doses of nitrogen oxides.

We carried out a three-month round-the-clock dynamic exposure of white male rats to nitrogen dioxide. Three groups of rats with 15 in each were used. The animals were kept on a standard ration. The first group of animals was exposed to 0.6 mg/m^3 NO_2, the second to 0.15 mg/m^3, and the third group was the control.

The round-the-clock dynamic exposure was carried out in chambers of 100 l capacity. We prepared nitrogen dioxide daily by Moser's method by heating Pb $(NO_3)_2$ with quartz sand in a retort at a temperature of about 223°C. A uniform penetration of NO_2 into the exposure chambers was achieved by means of a dispenser which we constructed, consisting of a time mechanism and a Luer syringe. The NO_2 content of the air of the exposure chambers was determined daily by the vacuum method, using the Griess-Ilosvay reagent. The sensitivity of the method was 0.05 γ per 3.5 ml.

The chronic influence of NO_2 on the body of the animals was evaluated from their general condition, weight dynamics, change of conditioned reflex activity, dynamics of indices of peripheral blood, activity of whole blood cholinestrerase, number of fluorescent* leucocytes, and also excretion of coproporphyrins with the urine.

The method of conditioned reflexes has found extensive application in the establishment of maximum permissible concentrations of noxious substances in atmospheric air (Yu. V. Novikov, N. F. Izmerov, M. I. Gusev, Ya. G. Dvoskin, P. P. Yakimchuk, M. I. Gusev and K. N. Chelikanov, and others). The conditioned reflexes were developed in L. I. Kotlyarevskiy's chamber by a rapid method described by Ya. G. Dvoskin (1961).

The reflex activity was studied in the first group in seven rats (0.6 mg/m^3 NO_2), in the second group in seven rats (0.15 mg/m^3 NO_2) and in the third group in six rats (control).

Changes in the conditioned reflex activity were observed in only three rats (Nos. 22, 24 and 28) of the first group. They were expressed in a statistically significant increase of the latent period to light (6th and 7th weeks of exposure) and to a bell (7th and 9th weeks of exposure). In addition, disturbances of higher nervous activity appeared in isolated cases of disappearance of reflexes to light and in phase states. Even before the end of exposure (in one to two weeks), the higher nervous activity of the animals returned to normal.

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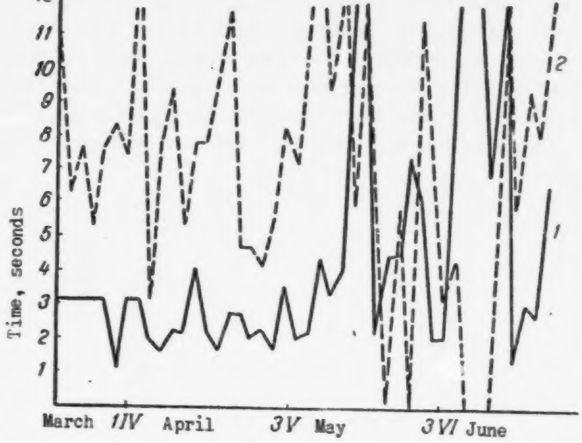

Fig. 1. Changes in the conditioned reflex activity in rat No. 24 (NO$_2$ concentration, 0.6 mg/m^3).
1 - response to a bell; 2 - response to light

As an illustration, we present graphs of the change of higher nervous activity in rats Nos. 24 and 30 (Figs. 1 and 2).

Examinations of the peripheral blood were performed every 10 days. The blood was taken from the rats' tails. In the blood of some rats of the first group, there was a tendency toward a decrease in the content of hemoglobin, a decline in the number of erythrocytes, and an increase in the number of leucocytes. No changes in the red and white counts of the peripheral blood were noted in rats of the second group.

The determination of whole blood cholinesterase activity in the rats was carried out by using a technique developed by A. A. Pokrovskiy (1953) and modified by A. P. Martynova (1957). The cholinesterase activity was determined every ten days. A total of twelve cholinesterase determinations were made in each group: once before the start of the experiment, nine times, in the course of exposure and twice during the recovery period. The cholinesterase activity was expressed as the number of seconds necessary for complete hydrolysis of acetylcholine. This time ranged from 32.6 to 52 seconds. However, no statistically reliable changes (increase or decrease) in the time of acetylcholine hydrolysis were noted.

As one of the tests, we used the luminescent analysis of leucocytes of peripheral blood. The determination was made once every ten days. The number of fluorescent leucocytes relative to their total number in the control ranged from 8.3 to 17.2%, in rats of the first group from 2.58 to 42.7%, and in rats of the second group from 6.2 to 33.2%. Among animals of the first group, statistically reliable changes were noted on the 40th, 50th and 60th days from the start of exposure. The confidence factor was 99.9%. Statistically reliable changes were also noted among rats of the second group at the end of the second month of exposure. We regarded this phenomenon as resulting from the action of random factors.

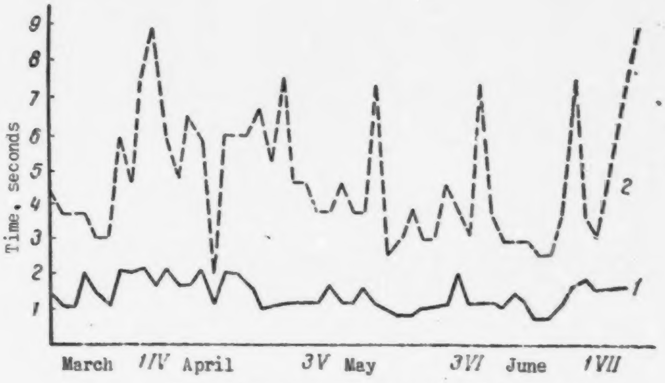

Fig. 2. Changes of conditioned reflex activity in rat No. 30 (NO_2 concentration, 0.15 mg/m^3). Notation same as in Fig. 1.

Table 1 gives the changes in the number of luminescent leucocytes under the influence of exposure.

We studied the change in the porphyrin metabolism of the rats under the influence of NO_2. In the practice of sanitary standardization of noxious substances in atmospheric air, the determination of coproporphyrin was first used by M. I. Gusev (1960), who found porphyrinuria during chronic exposure to lead oxide. G. I. Solomin (1961), T. M. Shul'ga (1961), Li Sheng (1962), P. P. Yakimchuk (1962), and others also investigated changes in the porphyrin metabolism during chronic exposures to noxious substances.

A six-month exposure of white rats to NO_2 in a concentration of 5.7 mg/m^3 for six hours a day gave a statistically reliable increase of coproporphyrin; a concentration of 0.84 mg/m^3 NO_2 caused a relative increase of coproporphyrins, although it was statistically unreliable (P. P. Yakimchuk, 1962).

In the present study, the urine of white rats was analyzed for coproporphyrins every seven days. The daily amount of urine was collected from the entire group of rats into a single sample. The content of coproporphyrins was

Table 1

Change in the Number of Fluorescent Leucocytes in the Blood of White Rats Under the Influence of a Three-Month Round-the-Clock Exposure to NO_2 (in percent).

Group	Background	Period of Exposure									Recovery	
	15/II	26/III	6/IV	16/IV	28/IV	9/V	21/V	31/V	11/VI	21/VI	3/VII	15/VII
Control	10,42	11,2	8,3	11,6	11,9	17,1	17,2	12,5	10,1	9,6	12,2	15,7
First group — 0,6 mg/m^3 NO_2	2,58	22,2	15,7	27	19,3	35,2	42,7	28	15,5	23,2	22	30,5
t	5,7	1,7	3,2	2,8	0,9	6,7	6,5	3,1	1,5	1,5	1,5	2,2
Second group — 0,15 mg/m^3 NO_2	7,85	12,35	8,14	20,2	6,2	23,7	33,2	The experiment was unsucessful	9,4	19,7	19,7	13,7
t	1,6	0,18	0,04	1,7	1,8	1,5	1,9		0,1	2,4	1,3	0,2

Table 2

Excretion of Coproporphyrin with the Urine in 24 Hours per Rat per 100 g of Body Weight, γ.

Index	Back-ground	Period of Exposure												Recovery		
	2/III	18/III	26/III	31/III	8/IV	15/IV	22/IV	3/V	13/V	20/V	27/V	4/VI	17/VI	25/VI	4/VII	12/VII
Control																
Amount of copro-porphyrins per rat	0,54	1,6	3,3	2,1	1,36	2,36	2,43	4,9	1,18	2,73	1,2	2,08	1,7	1,99	1,4	1,75
Calculation of coproporphyrin per 100 g of body weight	0,37	1,03	2,08	1,32	0,76	1,76	1,18	2,0	0,47	1,06	0,45	0,77	0,60	0,65	0,45	0,51
First Group (0,6 mg/m^3 NO$_2$)																
Amount of copro-porphyrins per rat	1,04	1,32	2,5	4,8	5,08	6,1	4,64	7,2	2,39	3,5	3,12	2,6	1,92	2,8	3	2,25
Calculation of coproporphyrin per 100 g of body weight	0,65	0,81	1,4	2,7	2,5	2,9	2,13	3,1	0,98	1,34	1,2	0,9	0,6	0,84	0,96	0,58
Second Group (0,15 mg/m^3 NO$_2$)																
Amount of copro-porphyrins per rat	1,08	1,75	1,79	2,14	2,2	4,15	2,5	4,66	1,06	2,97	0,67	2,58	1,94	1,93	1,1	1,97
Calculation of coproporphyrin per 100 g of body weight	0,63	1,10	1,10	1,30	1,23	2,18	1,21	1,55	0,43	1,10	0,25	0,94	0,66	0,62	0,35	0,59

determined by Fischer's method modified by Yu. K. Smirnov on an SF-4 spectrophotometer. A total of 16 experiments were carried out (one before, 10 during and three after the exposure). Analysis of the data obtained on the coproporphyrin metabolism shows an increase in the content of coproporphyrins in rats of the first group (exposure to a concentration of 0.6 mg/m^3 NO_2) from 1.04 to 7.2γ (average, 3.6γ). However, this increase was found to be unreliable.

Table 2 gives the results of the coproporphyrin study.

The relative increase of coproporphyrins was determined approximately starting with the third week; starting with the 8th week, it decreased and remained at the same level until the end of the experiment (12th week).

The coproporphyrin content in the urine of rats of the second group was almost the same as in the control animals, and ranged from 1 to 4.6γ (average 2.4γ). Treatment of these data showed these changes to be statistically unreliable.

Observation of the weight dynamics of the animals in the first and second groups did not yield any significant deviations as compared with the analogous indices in the control.

Histopathologic changes in internal organs were observed only at an NO_2 concentration of 0.6 mg/m^3. These changes were found chiefly in the lungs, in the form of catarrhal bronchitis, tracheitis, peribronchial pneumonia and symptoms of moderate pneumosclerosis.

Conclusions

1. A round-the-clock three-month exposure of rats to NO_2 in a concentration of 0.6 mg/m^3 caused a significant increase in the amount of fluorescent leucocytes, an increase in the latent period of response to light and to a bell, individual phase states, a partial disappearance of reflexes to light, and morphological changes in internal organs.

2. Exposure of the rats to NO_2 in a concentration of 0.15 mg/m^3 did not cause any organic or functional changes.

3. The mean daily maximum permissible concentration of NO_2 in atmospheric air may be recommended at the level of its highest single value, i. e., 0.085 mg/m^3.

LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1968 bibliography.

EXPERIMENTAL DATA FOR A SANITARY STANDARDIZATION OF LOW CONCENTRATIONS OF A MIXTURE OF FURFURAL, METHANOL AND HYDROLYTIC ETHANOL IN ATMOSPHERIC AIR

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The object of our work was to carry out an experimental study of the effect of furfural, methanol and hydrolytic ethanol, present either separately or together, on the body of man and animals. These substances are always found in atmospheric air around hydrolysis plants in Uzbekistan and may have an adverse effect on the health of the population. For this reason, the establishment of the maximum permissible concentrations of these substances in atmospheric air when present individually and together is timely and urgent.

Furfural ($C_5H_4O_2$) is a nerve poison; it affects the central nervous system and slightly irritates the mucous membranes (N. V. Lazarev, 1963) To determine furfural, we used the method of I. M. Korenman (1930), modified by us under the direction of M. V. Alekseyeva. The principle of the method lies in the fact that furfural reacts with aniline, forming furfuralaniline in the presence of acetic acid. The sensitivity of the method is 0.25 μg per 2 ml (R. Ubaydullayev, 1963). The highest single and mean daily maximum permissible concentrations of furfural in atmospheric air are 0.05 mg/m^3.

Methanol (CH_3OH) is a strong, chiefly nerve and vascular poison with a marked cumulative effect (its narcotic effect is of no major practical significance) (N. V. Lazarev, 1963).

Of the existing methods of determination of methanol, the most sensitive and widely employed is that of M. V. Alekseyeva (1963) based on the oxidation of methanol to formaldehyde by potassium permanganate. Formaldehyde is determined by the reaction with chromotropic acid, which gives a purple color. The method is specific in the absence of formaldehyde; the sensitivity of the method is 0.4 μg per 5 ml. The highest single maximum permissible concentration is 1 mg/m^3, and the mean daily concentration, 0.5 mg/m^3.

Ethyl alcohol (C_2H_5OH) is a narcotic causing excitation at first, and then at larger doses, paralysis of the central nervous system. Hydrolytic alcohol is obtained by alcoholic fermentation of sugars formed in the hydrolysis of wood pulp, vegetable waste, or in the sulfate digestion of cellulose (GOST 8314-57). Hydrolytic ethyl alcohol comes in brands A and B and is 95° proof. The impurities in this alcohol include acetic acids, unsaturated compounds, methyl alcohol, furfural, etc., which together amount to 0.2 and 0.5%.

N. V. Lazarev (1954) pointed out that hydrolytic ethyl alcohol is 1.3 times more toxic than the pure compound. However, our studies show that the thresholds of smell and of the effect of low concentrations of hydrolytic and pure ethyl alcohols on the electrical activity of the human brain are the same.

To determine ethanol in atmospheric air, we used the method of I. A. Selina (1962). The method is based on the reaction of the alcohol with a vanadium-hydroxyquinoline complex; an orange color is formed. The sensitivity of the method is 0.002 mg per 2.5 ml. The method is nonspecific, and alcohols interfere with the determination. For this reason, we had to determine the total alcohols (methyl, hydrolytic, and ethyl). Methanol was then determined by a specific method, and by subtracting it from the total alcohols, the concentration of ethyl alcohol is obtained. To make sure that the total concentration being determined was correct, we checked the methanol and ethanol concentrations, individual then total, for 3 to 4 days before each experiment, and only then did we start the experiment.

To substantiate the maximum permissible concentration in atmospheric air, use was made of the methods recommended by the Committee on Sanitary Protection of Atmospheric Air (V. A. Ryazanov, K. A. Bushtuyeva, and Yu. V. Novikov, 1957).

We began our study with the determination of the odor threshold of furfural, methanol and hydrolytic ethanol for each substance individually, and then for their combination. The threshold of odor perception for furfural was determined in 38 persons, for methanol in 25, and for hydrolytic ethanol in 25.

The odor threshold of furfural, methanol and hydrolytic ethanol present together was determined in 19 persons, the individual threshold of smell having been established for each substance. In all, 409 determinations were made.

Results of the study of thresholds of olfactory perception for furfural, methanol and hydrolytic ethanol vapors are listed in Table 1.

Table 1

Odor Thresholds of Furfural, Methanol and Hydrolytic Ethanol

No.	Subject	Individual Thresholds, mg/m^3			Combined Effect							
					Threshold Concentration				Subthreshold Concentration			
					mg/m^3			Sum of fractions of Individual thresholds	mg/m^3			Sum of Fractions of Individual Thresholds
		Furfural	Methanol	Hydrolytic Ethanol	Furfural	Methanol	Hydrolytic Ethanol		Furfural	Methanol	Hydrolytic Ethanol	
1	P.T.	1,02	7,5	8,70	0,52	2,92	3,79	1,34	0,46	3,02	3,40	1,24
2	Ya.Ye	1,02	5,5	7,30	0,46	2,58	2,81	1,30	0,39	1,8	2,70	1,06
3	T.R.	1,02	5,5	8,70	0,46	2,58	2,81	1,23	0,37	1,74	2,63	1,00
4	Kh.Z.	1,02	6,5	7,3	0,46	2,58	2,81	1,22	0,39	1,86	2,70	1,01
5	G.Ye.	1,43	6,5	8,0	0,46	3,02	3,40	1,20	0,50	2,46	3,14	1,11
6	Ch.G.	1,36	8,4	8,7	0,52	2,92	3,79	1,16	0,46	3,02	3,40	1,07
7	A.V.	1,36	10,3	10,2	0,56	2,92	5,17	1,16	0,52	3,11	4,01	1,07
8	B.Ye.	1,02	8,4	7,30	0,46	2,58	2,81	1,14	0,37	1,77	2,63	1,01
9	S.G.	1,32	7,5	7,1	0,50	2,46	3,14	1,13	0,48	2,46	2,98	1,09
10	R.T.	1,36	8,4	8,0	0,46	3,02	3,40	1,10	0,48	2,46	2,98	1,01
11	B.T.	1,36	8,4	8,7	0,46	3,02	3,40	1,08	0,50	2,46	3,14	1,01
12	K.M.	1,0	4,5	8,0	0,37	2,0	2,49	1,04	0,35	1,82	2,37	0,96
13	S.I.	1,0	5,4	7,1	0,37	2,0	2,49	1,09	0,35	1,82	2,37	1,01
14	M.L.	1,0	4,5	7,1	0,35	1,82	2.37	1,08	0,3	1,77	2,27	1,00
15	L.N.	1,0	4,5	7,1	0,35	1,82	2,37	1,08	0,36	0,73	2,27	1,00
16	K.R.	1,0	5,4	7,1	0,35	1,82	2,37	1,01	0,35	1,76	2,27	0,94
17	B.V.	1,04	6,5	7,1	0,37	1,83	2,60	1,01	0,37	2,0	2,20	0,95
18	Ch.A.	1,04	6,5	7,1	0,37	1,83	2,60	1,01	0,37	2,0	2,20	0,95
19	I.L.	1,14	7,5	8,0	0,40	2,50	2,70	1,01	0,38	1,80	2.65	0,90

As is evident from this table, the odor threshold of the mixture of furfural, methanol, and hydrolytic alcohol for eight of the most sensitive persons, with an index of total concentration expressed in fractions of the threshold values, is equal to 1.0-1.09. The threshold of olfactory perception is not the limit of the physiological activity of active stimulations. Very frequently, without being associated with the appearance of distinct perceptions, stimulations cause certain physiological reactions of so-called imperceptible character (G. V. Gershuni, 1948).

The light sensitivity of the eyes is a delicate and labile function of the visual analyser [trans.: system]. I. P. Pavlov (1952) treated the cerebral cortex as a set of analysers whose elements are in a constant mutual interaction. The focus of stimulation arising in the cerebral cortex as a result of stimulation of the olfactory receptor may radiate over the cerebral cortex, reaching the visual area, and thereby change the functional state of the visual center, increasing or decreasing the light sensitivity of the eye at the instant of dark adaptation. Hence, a change in the level of the light sensitivity of the eye under the influence of secondary stimuli should be regarded as a reflection of the physiological shifts of both peripheral and central origin.

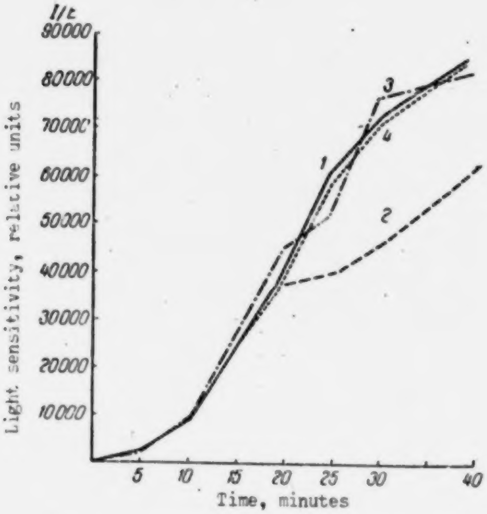

Fig. 1. Light sensitivity of the eye during combined inhalation of vapors of furfural, methanol and hydrolytic ethanol in subject I. L. 1 - pure air; 2 - mixture of furfural (0.16 mg/m^3), methanol (1.98 mg/m^3), and hydrolytic ethanol (2.56 mg/m^3); 3 - mixture of furfural (0.13 mg/m^3), methanol (1.55 mg/m^3), and hydrolytic ethanol (2.15 mg/m^3); 4 - mixture of furfural (0.11 mg/m^3), methanol (1.36 mg/m^3), and hydrolytic ethanol (1.62 mg/m^3).

The threshold of the reflex effect of furfural, methanol and hydrolytic ethanol on the light sensitivity of the eye during their combined presence was determined in three persons most sensitive to the odor threshold, after first establishing the individual threshold of the reflex effect of these substances on the light sensitivity of the eye. In all, three mixtures of the substances studied were investigated. The results of the tests are listed in Table 2 and Fig. 1.

The first combination of substances, 0.16 mg/m^3 of furfural, 1.98 mg/m^3 of methanol and 2.56 mg/m^3 of hydrolytic ethanol caused statistically reliable changes in the light sensitivity of the eye in all three subjects.

The second combination, 0.13 mg/m^3 of furfural, 1.55 mg/m^3 of methanol and 2.15 mg/m^3 of hydrolytic ethanol, caused significant changes in the course of the dark adaptation curve in only two subjects.

Table 2

Threshold of the Reflex Effect of Furfural, Methanol and Hydrolytic Alcohol on the Light Sensitivity of the Eye.

No.	Subject	Individual Threshold From Adaptometry			Combined Effect							
					Threshold Concentration				Subthreshold Concentration			
					mg/m^3			Sum of Fractions of Individual Thresholds	mg/m^3			Sum of Fractions of Individual Threshold
		Furfural	Methanol	Hydrolytic Ethanol	Furfural	Methanol	Hydrolytic Ethanol		Furfural	Methanol	Hydrolytic Ethanol	
1	B.V.	0,30	4,11	6,97	0,13	1,55	2,15	1,03	0,11	1,36	1,62	0,86
2	Ch.A.	0,30	3,53	6,97	0,13	1,50	2,15	1,09	0,11	1,36	1,62	0,91
3	I. L.	0,42	4,11	8,29	0,16	1,98	2,56	1,16	1,13	1,55	2,15	0,93

The sum of furfural, methanol, and hydrolytic ethanol, respectively 0.11 + 1.36 + 1.62 mg/m^3, had no effect on the light sensitivity of the eye.

Thus, for the most sensitive persons, the threshold of the reflex effect of methanol, furfural, and hydrolytic ethanol present together on the light sensitivity of the eye was at the level of the index of total concentration expressed in fractions of the threshold values of each component part, 1.03.

In recent years, the electroencephalographic method has found broad and justified applications in the experimental standardization of atmospheric pollutants (K. A. Bushtuyeva et al., 1960; V. A. Gofmekler, 1961; R. Ubaydullayev, 1961; A. D. Semenenko and B. N. Balashev, 1963; Yu. Ye. Korneyev, 1965; U. G. Pogosyan, 1965, and others). This method makes it possible to identify the changes arising directly in the cortical cells of the brain and constitutes a highly sensitive indicator of the functional state of the nervous system.

In our studies, we used two modifications of this method. The first was quantitative analysis of the alpha rhythm burst response (A. D. Semenenko and B. N. Balashev, 1963), and the second was the development of the electrocortical conditioned reflex (K. A. Bushtuyeva, A. D. Semenenko and Ye. F. Polezhayev, 1960).

The electric potentials were recorded on an eight-channel electroencephalograph of the Orion Budapest Co.

The change of the alpha rhythm during inhalation of the mixture studied was studied on six persons. For two weeks, they were subjected to training experiments in order to obtain a well-defined alpha rhythm and a definite dynamic stereotype. The main observations were started after data were obtained which fluctuated comparatively little during inhalation of pure air; this was accomplished by using stable, standard observation conditions.

The texts were carried out in the following order: at first, the initial background of electric potentials was recorded while pure air was being supplied; then, starting with the fourth minute the subject breathed the mixture studied (for six minutes), and during the next 6 minutes (period of the aftereffect), pure air was supplied again. The entire observation lasted 18 minutes, including 3 minutes for training. In the course of each minute, rhythmic light was supplied for 18 seconds, and its intensity was changed every 5 seconds. In the intervals separating the switching on of the light, the subject limbered up, then a sound of variable intensity was turned on for 10 seconds. After the sound was discontinued, the subject waited for the light for 7 seconds.

In the course of the entire study, the subject was in a state of active mental and motor activity. This removed the possibility of the onset of phase states.

The biopotentials were recorded by the bipolar method from the temporal-occipital areas of both cerebral hemispheres. The electroencephalograms were analyzed from the integrated energy of the amplified rhythm of the brain.

The experimental data were treated statistically according to periods (of two minutes each), and the confidence factor of the shifts obtained was found. Results of quantitative analysis of the human reaction of burst of the alpha rhythm during inhalation of furfural, methanol, and hydrolytic ethanol vapors present together are listed in Table 3.

These data show that a mixture containing 0.026 mg/m^3 furfural, 0.50 mg/m^3 methanol, and 1.75 mg/m^3 hydrolytic ethanol, with a 0.99 index of the total concentration expressed in fractions of the threshold value based on the electroencephalography for each component part, causes statistically reliable changes of the amplified intrinsic rhythm in all six subjects. Fig. 2 shows the curves obtained with subject L. N.

For one of the most sensitive persons, the active concentration of the mixture of furfural (0.020 mg/m^3), methanol (0.38 mg/m^3), and hydrolytic ethanol (1.70 mg/m^3) amounted to 0.85 in fractions of the threshold values based on the electroencephalography. The inactive concentration for all the subjects, expressed in fractions of the thresholds based on the electroencephalography was equal to 0.78. Thus, the threshold of the combined effect of furfural, methanol, and hydrolytic ethanol on the electric activity of

Table 3

Study of the Electrical Activity of the Brain by the Method of Quantitative Analysis of the Alpha Rhythm Burst Response

Threshold		Substance	Subject					
			S.I.	K.R.	M.L.	K.M.	L.N.	L.T.
Individual Thresholds for Single Substances	mg/m3	Furfural	0,078	0,078	0,078	0,078	—	—
		Methanol	1,17	1,46	1,46	1,46	—	—
		Hydrolytic Ethanol	6,14	6,14	6,14	6,10	—	—
Threshold Combinations	mg/m3	Furfural	0,020	0,026	0,026	0,026	0,026	0,026
		Methanol	0,38	0,50	0,50	0,50	0,50	0,50
		Hydrolytic Ethanol	1,70	1,96	1,96	1,96	1,96	1,95
		Sum of Fractions of Individual Threshold	0,85	0,99	0,99	0,99		
Subthreshold Combinations	mg/m3	Furfural	0,019	0,020	0,020	0,020	0,020	0,020
		Methanol	0,35	0,38	0,38	0,38	0,38	0,38
		Hydrolytie Ethanol	1,60	1,70	1,70	1,70	1,70	1,70
		Sum of Fractions of Individual Threshold	0,79	0,78	0,78	0,78		
Sum of Fractions of Maximum Permissible Concentration		Active	1,12	1,40	1,40	1,40	1,40	1,40
		Inactive	1,05	1,42	1,12	1,12	1,12	1,12

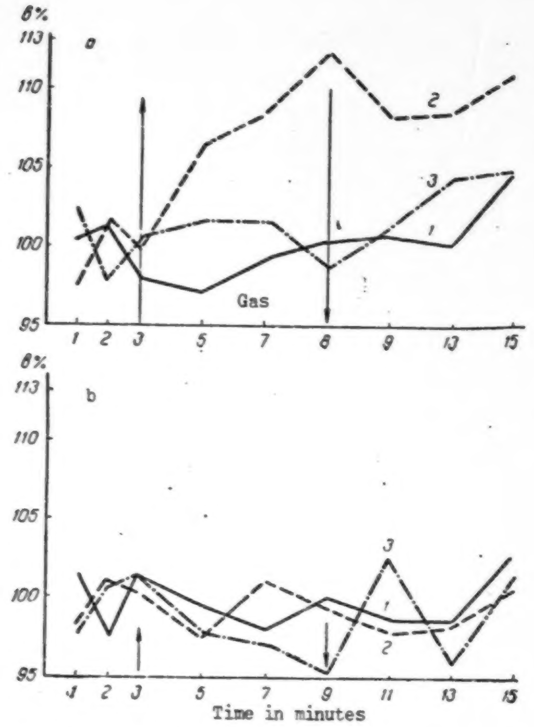

Fig. 2. Amplitudes of quantitative analysis of the alpha rhythm burst response during combined inhalation of vapors of furfural, methanol, and hydrolytic ethanol in subject L. N.

a - left hemisphere; b - right hemisphere; 1 - pure air; 2 - mixture of furfural (0.026 mg/m^3), methanol (0.50 mg/m^3), and hydrolytic ethanol (1.96 mg/m^3); 3 - mixture of furfural (0.020 mg/m^3), methanol (0.38 mg/m^3), and hydrolytic ethanol (1.70 mg/m^3).

the brain for the most sensitive persons, expressed as the sum of parts of the thresholds based on the electroencephalography, amounts to 0.85. Concentrations with a total index of 0.78 and lower have no effect on the electrical activity of the brain.

As already noted, we also used the electrocortical conditioned reflex method in our studies.

Four persons aged 20 to 30 years with a normal sense of smell and a distinct alpha rhythm were chosen for the study. The subjects were the most sensitive from the standpoint of the odor threshold of smell and effect of furfural, methanol, and hydrolytic ethanol, both separately and in combination, on the electrical activity of the cerebral cortex as determined by the method of quantitative analysis of the alpha rhythm burst response.

Two combinations of furfural, methanol, and hydrolytic ethanol were studied. The first consisted of 0.020, 0.37 and 1.68 mg/m^3 respectively. The index of the sum in fractions of the maximum permissible concentration for isolated action amounted to 1.11.

Second combination: 0.016 mg/m^3 furfural, 0.30 mg/m^3 methanol, and 1.50 mg/m^3 hydrolytic ethanol. The index of the sum in fractions of the maximum permissible concentration was 0.92.

The results of the study are listed in Table 4 and in Figs. 3-5.

Table 4

Study of the Electrical Activity of the Brain by the Method of Development of the Electrocortical Conditioned Reflex.

Indices	Substances	Subject			
		I.S.	K.R.	K.M.	L.N.
Threshold Combination mg/m^3	Furfural	0,020	0,020	0,020	0,020
	Methanol	0,37	0,37	0,37	0,37
	Hydrolytic Ethanol .	1,73	1,73	1,73	1,73
	Sum of Fractions of Maximum Permissible Concentration . . .	1,11	1,11	1,11	1,11
Subthreshold Combination in mg/m^3	Furfural	0,016	0,016	0,016	0,016
	Methanol	0,30	0,30	0,30	0,30
	Hydrolytic Ethanol .	1,50	1,50	1,50	1,50
	Sum of Fractions of Maximum Permissible Concentration . . .	0,92	0,92	0,92	0,92

According to the data of Table 4 one can state that the mixture with the total concentration index of 1.11 was active for all the subjects, and the sum of fractions of 0.92 was found to be inactive.

The results of the studies were treated statistically: in collaboration with V. A. Gofmekler, a method was proposed for the numerical treatment of the electroencephalographic curves; the latter were then studied for changes under the influence of inhalation of the combination of the substances studied.

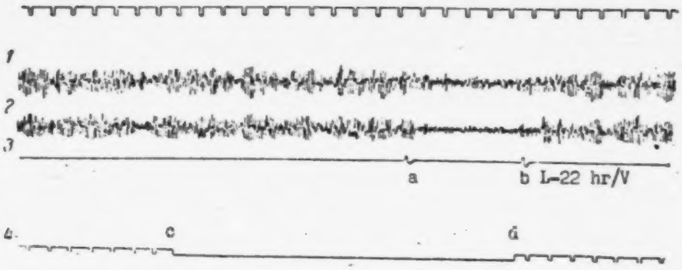

Fig. 3. Electrocortical conditioned reflex during inhalation of pure air in subject L. N., 22nd pairing.
1 - left temporal-occipital lead; 2 - right temporal-occipital lead; 3 - mark of light supply (a-b); 4 - mark of gas supply (c-d).

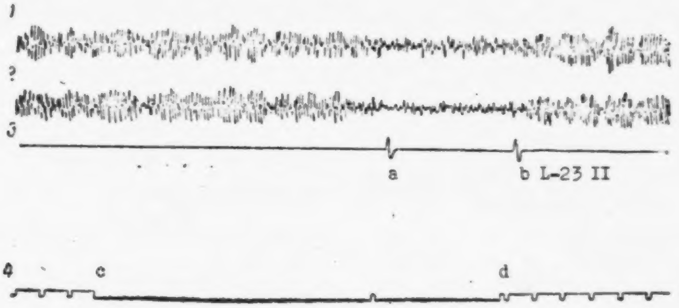

Fig. 4. Electrocortical conditioned reflex during combined inhalation of vapors of furfural (0.016 mg/m^3), methanol (0.30 mg/m^3), and hydrolytic ethanol (1.50 mg/m^3) in subject L. N., 23rd pairing. Notation same as in Fig. 3.

Thus, of the two modifications of the electroencephalographic method of investigation employed, the more sensitive and convenient for a statistical treatment was found to be the method of development of the electrocortical conditioned reflex.

The investigations performed, which involved three physiological tests, show that low concentrations of the mixture of furfural, methanol, and hydrolytic ethanol manifest their influence in the most sensitive persons in accordance with the principle of simple summation.

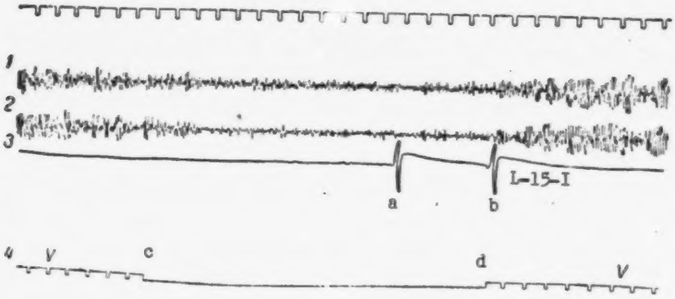

Fig. 5. Electrocortical conditioned reflex during combined inhalation of vapors of furfural (0.020 mg/m^3), methanol (0.37 mg/m^3), and hydrolytic ethanol (1.73 mg/m^3) in subject L. N., 15th pairing. Notation same as in Fig. 3.

The data obtained lead to the conclusion that during the combined presence of furfural, methanol and hydrolytic ethanol in atmospheric air, the sum of their concentrations expressed in fractions of the maximum permissible values for isolated action should not exceed unity.

In order to determine the effect of low concentrations of a mixture of furfural, methanol, and hydrolytic ethanol for the purpose of substantiating the mean daily maximum permissible concentration in atmospheric air, a chronic round-the-clock experiment was performed on the animals. White rats weighing 130-140 g were used; the exposure lasted 90 days. Rats of group 1 were exposed to the action of a mixture of furfural, methanol, and hydrolytic ethanol in a concentration at the level of the minimum active value for each substance taken separately, i.e., a concentration 20 times the recommended highest single maximum permissible concentration for their combined presence. On the average, the concentration was 0.3 ± 0.018 mg/m^3 furfural, 5.79 ± 0.65 mg/m^3 methanol, and 28.48 ± 1.52 mg/m^3 hydrolytic ethanol. The total concentration index, expressed in fractions of the mean daily maximum permissible values for isolated action, was equal to 23.27.

Rats of group II were exposed to the action of the mixture in a concentration corresponding to the level of the existing mean daily maximum permissible concentrations. On the average, it was 0.053 ± 0.0045 mg/m^3 furfural, 0.54 ± 0.045 mg/m^3 methanol, and 5.88 ± 0.47 mg/m^3 hydrolytic ethanol, with a total concentration index of 3.31.

Rats of group III were exposed to the action of 0.021 ± 0.0012 mg/m^3 furfural, 0.18 ± 0.018 mg/m^3 methanol, and 2.0 ± 0.12 mg/m^3 hydrolytic ethanol. The total concentration index expressed in fractions of the mean daily maximum permissible values for each substance for isolated action was 1.18.

Rats of group IV were the controls. The experimental mixture of air with the specified amount of the substances studied was supplied to the chamber at a rate of 35 ℓ per minute. The concentration of the mixture was determined daily in each chamber.

In order to evaluate the toxic effect of the furfural-methanol-hydrolytic ethanol mixture, the following factors were studied on the experimental animals during the course of the entire experiment: general state, behavior and weight dynamics of the animals, ratio of chronaxies of antagonist muscles, whole blood cholinesterase, protein fractions of the blood serum, excretion of coproporphyrin with the urine, and histopathologic changes in internal organs after completion of the exposure.

The resorptive effect of various noxious substances in concentrations found in atmospheric air included general, nonspecific, and sometimes insignificant shifts. As a rule, these shifts are functional in character. They are based on reflex and defense reactions, and their chief manifestation is protective inhibition (K. A. Bushtuyeva, 1964). The participation of central nervous mechanisms in the generation of these shifts should be considered general and mandatory. The tests that we used were designed to reveal shifts that are not characteristic or specific for a given substance, but have a general biological importance.

In the study of the behavior and state of the animals in the course of the entire experiment, no symptoms were observed which might have indicated an irritation of the mucous membranes or clinical disturbances of the nervous system. The animals were weighed every 20 days. There were no appreciable weight changes in any of the groups as compared with the control.

Experimental studies by many authors indicate that one of the mechanisms by which the relationships between the nerve centers and peripheral formations are manifested in subordination. The interruption of the subordinating influence of the central nervous system immediately affects the level of many functional characteristics of the neuromuscular apparatus, particularly its excitability, one of the indices of which is chronaxy. Normally, the ratio of the chronaxies of extensors and flexors is greater than unity. Under the influence of various chemical ingredients, this ratio approaches unity or falls below it; in other words, the chronaxies of antagonist muscles converge, or a distortion of their ratio takes place. This shows a weakening of the subordinating influence of the central nervous system, caused by the development of an inhibition process in the brain (V. A. Ryazanov, 1964; N. A. Magnitskiy, 1937; Yu. M. Uflyand, 1941, and others).

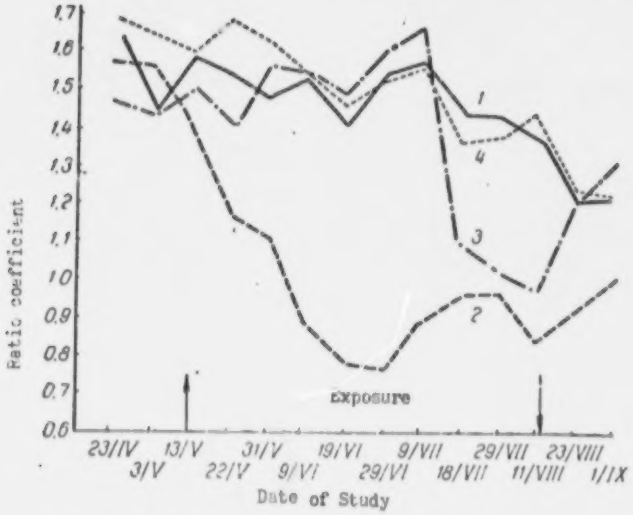

Fig. 6. Ratio of chronaxies of antagonist muscles in rats during combined inhalation of vapors of furfural, methanol, and hydrolytic ethanol.

1 - control group; 2 - first group; 3 - second group; 4 - third group

In our studies, the motor chronaxy of antagonist muscles was determined in 5 rats of each group at one and the same time and under the same conditions, using a domestic ISE-01 pulsed stimulator.

Results of the study by groups and graphic illustrations are shown in Table 5 and Fig. 6.

Data from the study of the chronaxy of antagonist muscles have shown that in rats of group I in the 4th week of exposure, a disturbance of the normal chronaxial ratio takes place. These changes were statistically reliable until the end of the exposure period. They were less pronounced in rats of group II; only by the end of the 3rd month did a disturbance occur in the normal chronaxial ratio of antagonist muscles, and the results of the study became statistically reliable. In rats of group III, no changes in the chronaxial ratio of antagonist muscles were observed as compared with the control group.

One of the processes of transfer of nerve impulses is closely related to the liberation of acetylcholine at nerve endings, which permits the transfer of the impulses from nerve endings to the receptor structure. The enzyme that splits acetylcholine is cholinesterase.

Table 5

Ratio of Chronaxy of Extensor and Flexor Muscles in Rats During Inhalation of Furfural, Methanol, and Hydrolytic Ethanol in Combination.

Periods of Experiment	Date	Group			
		I	II	III	Control
Before Exposure	20/IV 1954 r.	1,59	1,47	1,69	1,65
	3/V	1,56	1,40	1,65	1,45
	13/V	1,40	1,50	1,60	1,58
Exposure	22/V	1,18	1,41	1,69	1,54
	31/V	1,11	1,57	1,62	1,48
	9/VI	0.88(c)	1,55	1,54	1,53
	19/VI	0,79(c)	1,50	1,46	1,41
	29/VI	0,77(c)	1,60	1,53	1,54
	9/VII	0,88(b)	1,67	1,57	1,57
	18/VII	0,96(b)	1,11	1,37	1,44
	29/VII	0,97(a)	1,02	1,39	1,44
	11/VIII	0,84(c)	0,99(a)	1,45	1,39
Recovery Period	23/VIII	0,93	1,21	1,25	1,22
	1/IX	1,00	1,32	1,23	1,23

Note. Confidence factor: a - 95%, b - 99%, c - 99.9%.

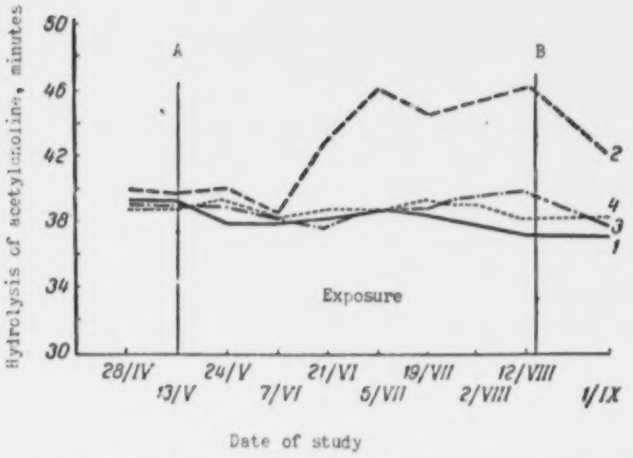

Fig. 7. Activity of whole blood cholinesterase in rats during combined inhalation of vapors of furfural, methanol, and hydrolytic ethanol. Notation same as in Fig. 6.

A change in the activity of cholinesterase and of the cholinergic reaction of the blood is one of the biochemical indicators of a disturbance of nervous activity (D. Ye. Al'pern, 1958). A decrease in cholinesterase activity should be considered in relation to disturbances of the activity of the central nervous system and to the development of inhibition in the cerebral cortex.

A study of the cholinesterase activity of whole blood was carried out by using a colorimetric method developed by A. A. Pokrovskiy (1953) and modified by A. P. Martynova (1957). The method is based on the determination of the time necessary for a change of the indicator as a result of the pH shift taking place during hydrolysis of acetylcholine.

The cholinesterase activity was determined in five rats of each group. The blood was taken from the tail vein once every 15 days, all the rules of asepsis being observed.

Results of changes in the cholinesterase activity of the whole blood of rats are listed by groups in Table 6 and Fig. 7.

Table 6

Results of Change of Cholinesterase Activity in Rats During Inhalation of a Mixture of Furfural, Methanol, and Hydrolytic Ethanol (Time, Minutes).

Periods of Experiment	Date	Group			
		I	II	III	Control
Before Exposure	28/IV 1964 r.	40,0	39,0	38,8	39,2
	13/V	39,0	39,0	39,0	39,2
Exposure	24/V	40,0	38,8	39,2	38,0
	7/VI	38,4	38,2	38,2	38,0
	21/VI	43,0(b)	37,8	38,6	38,2
	5/VII	46,2(c)	38,8	38,8	38,8
	19/VII	44,8(c)	39,2	39,2	38,6
	2/VIII	45,4(c)	39,6(a)	39,2	38,0
	12/VIII	46,4(c)	40,0(a)	38,6	37,6
Recovery Period	1/IX	42,4(b)	38,0	38,5	37,5

Note. Confidence factor: a - 95%; b - 99%; c - 99.9%.

As is evident from Table 6 and the graph, at the end of the first month from the start of exposure, a depression of the activity of blood cholinesterase took place in animals of group I, and the duration of acetylcholine hydrolysis increased to 43 minutes from the initial level (39-40 minutes), whereas at the end of the 2nd and 3rd months, it reached 45-46 1/2 minutes.

The depression of cholinesterase activity in rats of group II was more moderate. A significant result was obtained only twice toward the end of exposure. No changes were observed in animals of group III or in the controls.

The decrease in cholinesterase activity observed in our studies indicates a nonspecific protective response of compensatory character.

Chronic exposure of white rats to vapors of furfural, methanol, and hydrolytic ethanol (group I) causes changes in the protein fractions of the blood serum starting with the fourth week. At this time there was a sharp increase of the globulin fraction and decrease of the albumin fraction. The results of the study were statistically reliable (Table 7).

In rats of groups II-III, no changes were observed in the composition of the protein fractions as compared with the control group. No changes were established in the total amount of protein in any of the groups of animals studied.

Table 7

Changes in the Ratio of the Albumin-Globulin Fraction of the Blood Serum in Rats during Inhalation of a Mixture of Furfural, Methanol, and Hydrolytic Ethanol Vapors.

Periods of Experiment	Date	Group			
		I	II	III	Control
Before Exposure	27/IV 1964 r.	0,84	0,72	0,71	0,76
	13/V	0,89	0,75	0,83	0,83
Exposure	26/V	0,75	0,66	0,73	0.76
	10/VI	0,44(a)	0,61	0,66	0.66
	23/VI	0,57	0,68	0,72	0.71
	7/VII	0,51(b)	0,73	0,72	0.77
	21/VII	0,47(a)	0,61	0,70	0.70
	13/VIII	0,50(a)	0,61	0,81	0.76
Recovery Period	12/IX	0,69	0,85	0,69	0,80

Note. Confidence factor: a - 95%, b - 99%.

Studies made by K. A. Bushtuyeva (1964), R. Ubaydullayev (1961), V. A. Chizhikov (1964), and P. G. Tkachev (1964) indicate that different chemical substances cause a uniform reaction in the protein fractions, chiefly characterized by a decrease in the percent content of albumins and an increase in the percent content of the globulin fraction, particularly beta globulins. Therefore, the changes obtained must not be attributed to a specific manifestation of the adverse effect of the substance studied. They should be ascribed to nonspecific compensatory shifts, which attest to a strain in the protective mechanisms of the animal organism.

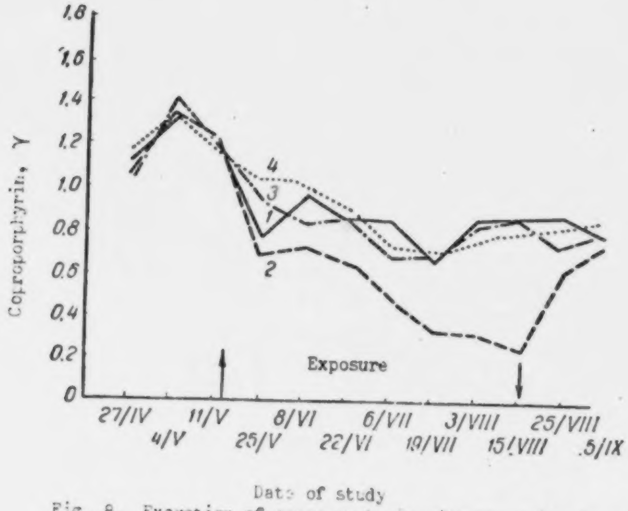

Fig. 8. Excretion of coproporphyrin with the urine in rats during combined inhalation of furfural, methanol, and hydrolytic ethanol vapors. Notation same as in Fig. 6.

Experimental studies by Yu. K. Smirnov (1953) show that the porphyrin metabolism is one of the indices reflecting the functional state of the central nervous system: an enhancement of porphyrin metabolism takes place upon stimulation of the hypothalamus region of the brain. Inhibitory processes in the brain change its functional activity and lead to a decrease in the porphyrin metabolism. That is why this test has been successfully used in recent years for the sanitary standardization of atmospheric pollutants (M. I. Gusev, 1960; G. I. Solomin, 1961, and others).

Coproporphyrin was determined once every two weeks; the results were recalculated for 100 g of body weight. In rats of group I, the excretion of coproporphyrin with the urine, in the second week of exposure, decreased sharply and remained at this level until the end of exposure. By the end of the recovery period, the excretion of coproporphyrin had returned to normal.

In rats of groups II and III, no statistically reliable changes were observed as compared with the control group (Table 8, Fig. 8).

Table 8

Excretion of Coproporphyrin with the Urine in Rats During Inhalation of Furfural, Methanol, and Hydrolytic Ethanol Vapors Under Chronic Experimental Conditions (in gammas per 100 g of body weight)

Period	Date	Group			
		I	II	III	Control
Before Exposure	27/IV 1964 r.	1,05	1.04	1,03	1,09
	4/V	1,36 (o)	1,42 (o)	1,33 (o)	1,34 (o)
	11/V	1,27	1,23	1,21	1,22
Exposure	25/V	0,68	0,96	1,06	0.80
	8/VI	0,72 (a)	0,86 (o)	1,00 (o)	0,98 (o)
	22/VI	0,67	0,86	0.89	0,88
	6/VII	0,48	0,72	0,77	0,85
	19/VII	0,36 (b)	0,70 (o)	0,70 (o)	0,68 (o)
	3/VIII	3.33	0,81	0,75	0,85
	15/VIII	0,26	0,85	0,79	0,87
Recovery Period	24/VIII	0.65	0,77	0,85	0,88
	4/IX	0,78 (o)	0,82 (o)	0,85 (o)	0,83 (o)

Note. Confidence factor: a - 95%; b - 99%, o - unreliable.

At the end of the exposure, 4 rats from each group were killed for histopathologic and pathomorphologic analyses.

The combined inhalational action of 0.3 mg/m^3 furfural, 5.79 mg/m^3 methanol, and 28.48 mg/m^3 hydrolytic ethanol in rats of group I causes an impairment of the blood circulation characterized by venous hyperemia with the appearance of a slight perivascular and pericellular edema in the brain and perivascular edema in the lungs and liver. There was a decrease in the reaction for ribonucleic acid in the brain cells and in the reaction for glycogen in the liver, with the onset of lipodystrophy in the latter; the function of the adrenal cortex decreased.

In rats of group II exposed to a mixture of 0.053 mg/m^3 furfural, 0.54 mg/m^3 methanol, and 5.83 mg/m^3 hydrolytic ethanol, a slight venous plethora of the liver, kidneys, and heart, and in some cases a slight dystrophic change of the liver were found.

In rats of group III exposed to the action of a mixture of 0.021 mg/m^3 furfural, 0.18 mg/m^3 methanol, and 2 mg/m^3 hydrolytic ethanol, no visible pathomorphologic changes were observed as compared with the control group.

Thus, a three-month chronic round-the-clock exposure of animals to a mixture of furfural, methanol, and ethanol affects the organism of white rats of group I by changing the motor chronaxy of antagonist muscles, cholinesterase activity of whole blood, excretion of coproporphyrin with the urine, and protein fractions of the blood serum, and affects histopathologic changes.

In rats of group II, the changes were very slight and were observed in the ratio of motor chronaxies of antagonist muscles, in the cholinesterase activity, and in histopathologic changes. No physiological, biochemical or histopathologic changes were found in rats of group III as compared with the control.

The experimental results lead to the conclusion that during the combined presence of furfural, methanol, and hydrolytic ethanol in atmospheric air, the sum of their concentrations expressed in fractions of the maximum permissible mean daily values for isolated action should not exceed unity.

Conclusions

1. During the combined action of furfural, methanol, and hydrolytic ethanol on the human body, a simple summation of the effect was established. This was confirmed by: a) the odor threshold of the combination, equal to 1.0 as the sum of parts of the corresponding odor thresholds for isolated action; b) the threshold of the reflex effect of the mixture on the light sensitivity of the eye, equal to 1.03 as the sum of parts of the threshold concentrations for isolated action; c) the threshold of the reflex effect of the mixture on the electrical activity of the brain, determined by quantitative analysis of the alpha rhythm burst response, equal to 0.85-0.99 as the sum of fractions of the threshold concentrations for isolated action, and the analogous threshold obtained by the electrocortical conditioned reflex method, equal to 0.78-0.84 as the sum of parts of the threshold concentrations for isolated action.

2. During the combined action of a mixture of furfural, methanol, and hydrolytic ethanol, the index of their total highest single maximum permissible concentration in atmospheric air should not exceed unity.

3. A study of the resorptive action of a mixture of furfural, methanol, and hydrolytic ethanol in the course of chronic round-the-clock exposure of white rats established the following: a) the sum of fractions of the mixture of furfural, methanol, and hydrolytic ethanol, equal to 23.2 of the existing mean daily maximum permissible concentrations for each of them taken separately, caused distinct changes as determined by all of the tests employed (chronaxy of antagonist muscles, cholinesterase activity, excretion of

coproporphyrin with the urine, protein fractions of the blood serum and histopathologic changes in the organs of the animals); b) at the end of the exposure, the sum of the fractions equal to 3.3 caused less distinct but statistically significant changes in the chronaxy of antagonist muscles, in cholinesterase activity, and also in the histopathologic picture of internal organs; c) the sum of fractions equal to 1.18 did not cause any changes in the body of the animals as compared with the control group.

4. On the basis of the fact that the mean daily maximum permissible concentration of atmospheric pollutants should be below the threshold of nonspecific shifts caused by the protective reaction of the body, one can recommend a mean daily concentration at the level of unity for a mixture of furfural, methanol, and hydrolytic ethanol in parts of the existing maximum permissible concentrations for isolated action.

LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1968 bibliography.

THRESHOLD CONCENTRATIONS OF DIKETENE DURING SHORT-TERM INHALATION

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 11, Izdatel'stvo "Meditsina" Moskva, p. 188-195, (1968).

Diketene ($C_4H_4O_2$) is a colorless, nonhygroscopic liquid with a pungent odor; it has a specific gravity of 1.09, and boils at 127.4 C. (N. V. Lazarev, 1963).

Diketene is finding increasingly broader applications in industry in the production of acetoacetic acid and its complex esters, dyes, pharmaceutical preparations, solvents, anticorrosive substances, plasticizers, etc.

The toxic effect of diketene has been insufficiently studied. Wooster et al. (1949) carried out a 10-minute exposure of mice, rats, rabbits and guinea pigs to diketene in a concentration of 0.67 mg/l. This concentration was tolerated by all the animals except the guinea pigs, for which it proved to be completely fatal. Emphysema and edema of the lungs were observed in the dead animals.

Of considerably greater interest are the studies of S. L. Danishevskiy (1948, 1950, 1951), dealing with the toxicology of diketene as an occupational poison. The authors subjected cats, rabbits, guinea pigs and white rats to a static two-hour exposure to diketene. The effect of concentrations from 0.108 to 7 mg/l was studied.

Danishevskiy notes that the irritant action of diketene was clearly manifested during the experiments. Convulsive twitches of the entire body were frequent. Death occurred 2-8 days after the exposure. Characteristic changes in the respiratory tract in acute poisoning are purulent-necrotic rhinotracheobronchitides with necrotizing pneumonic foci.

During chronic poisoning of rabbits and guinea pigs with diketene in a concentration of 0.5 mg/l for two hours a day, Danishevskiy observed changes in the respiratory function, weight loss, decrease in hemoglobin content, and increase in the number of leucocytes. All the rabbits died on the 13th-25th day of exposure. The guinea pigs were killed after 36-40 intoxications. The picture of chronic poisoning is characterized by the development of rhinotracheitis and pleuropneumonia with replacement of the inflammatory exudate by connective tissue. In addition, there were limited abscesses in the lungs, and occasionally a fibrous-purulent pericarditis that apparently formed shortly before the death of the animals.

Danishevskiy also studied the irritant effect of diketene on the human organism. The experiments were carried out on ten persons who inhaled diketene vapors through a gas mask connected to the gas chamber by a hose. A concentration of 0.003 mg/l during a one-minute exposure had a slight irritant effect in seven subjects, and 0.002 mg/l, in three subjects. The effect of lower concentrations was not studied by the author.

In people, the irritant effect of diketene shows up chiefly in the mucous membrane of the eyes, and this is followed by symptoms of irritation in the throat and nose.

On the basis of the threshold of irritant action, Danishevskiy tentatively proposed 0.5-1 mg/m^3 as the maximum permissible concentration of diketene in the air of work areas.

We have not found any other studies dealing with the toxicology of diketene in the accessible literature. The above-mentioned investigations deal with the biological effect of comparatively high diketene concentrations. In the air of populated areas, however, impurities are usually present in lower concentrations that may be biologically active.

The increase in the production and consumption of diketene and the consequent possibility of atmospheric pollution, as well as the lack of data on the threshold of the reflex effect of low diketene concentrations on the human organism prompted us to carry out a study for the purpose of substantiating the highest single maximum permissible concentration of this substance in atmospheric air.

Diketene was determined by a method developed by N. V. Stepanyuk, A. I. Bulycheva, P. A. Mel'nikova, and L. A. Grebennikova (1964). The method is based on the fact that the product of the reaction of diketene with sulfuric acid, by reacting with resorcinol under ultra-violet radiation, gives off a bluish-violet fluoresence. The sensitivity of the method is 0.01 μg per ml of analyzed solution. Ketene interferes with the determination, since it gives a similar reaction with sulfuric acid and resorcinol, but it was not used in our studies.

In working out the highest single maximum permissible concentration of diketene in atmospheric air, we first determined the threshold of its olfactory perception by using a standard technique (V. A. Ryazanov, K. A. Bushtuyeva, and Yu. V. Novikov, 1957). The observations were made on 20 persons ranging in age from 16 to 41 years. Seven diketene concentrations ranging from 3.333 to 0.011 mg/m^3 were studied, and 833 determinations were made. The results of the study are listed in Table 1.

As is evident from this table, the threshold of olfactory perception of diketene in the subjects ranged from 0.019 to 0.04 mg/m^3, and the subthreshold concentrations, from 0.011 to 0.03 mg/m^3. Thus, in the four most sensitive persons, the odor threshold of diketene was 0.019 mg/m^3, and the maximum imperceptible concentration was 0.011 mg/m^3.

Table 1

Data for the Determination of the Odor Threshold of Diketene.

Number of Subjects	Concentration, mg/m^3	
	Minimum Perceptible	Maximum Imperceptible
12	0,04	0,03
4	0,03	0,019
4	0,019	0,011

The next part of the study dealt with the reflex effect of low diketene concentrations on the light sensitivity of the eye. The observations were made on an ADM model adaptometer on four persons who proved to be the most sensitive in the determination of the odor threshold. The subjects ranged in age from 25 to 29 years. They all had a normal acuity of vision and smell.

The light sensitivity was determined every five minutes in the course of 30 minutes. Pure air (for the purpose of plotting the initial dark adaptation curve) and different diketene concentrations were supplied in the 16th minute of adaptation in the course of 4½ minutes.

The study showed that in two out of four subjects, the minumum active concentrations (0.021 and 0.02 mg/m^3) were close to the threshold values for smell, and the inactive concentrations (0.011 mg/m^3) coincided with the subthreshold ones for olfactory perception. In the remaining persons, lower thresholds of the effect were detected (0.011 mg/m^3), which corresponded to the level of the highest imperceptible concentration for smell. The inactive concentration was found to be 0.009 mg/m^3. An illustration is given in the graph of the light sensitivity of the eye for subject M. N. (Fig. 1).

The changes in the light sensitivity of the visual system amounted in all cases to an increase in the level of the dark adaptation curve and were statistically reliable.

Numerous studies carried out in recent years in the area of sanitary standardization of atmospheric pollutants have demonstrated the very high sensitivity of the method of studying the electrical activity of the brain in response to the action of various chemical substances. In consideration of this fact, we studied the influence of microconcentrations of diketene on the biocurrents of the brain in four persons, using the method of development of the electrocortical reflex recommended by K. A. Bushtuyevea et al. (1960). The subjects ranged in age from 16 to 35 years. Before the experiments, their odor threshold of diketene was determined, and in addition, the effect of diketene on the light sensitivity was studied in one subject.

The work was done with a Hungarian Orion electroencephalograph. The experiment was performed four to six times with each concentration. The gas (conditioned stimulus) was supplied for 15 seconds, and continuous light (unconditioned reflex) was used in the last 5 seconds of the action of the gas. The development of the conditioned reflex was evaluated from the desynchronization of the alpha rhythm before the light was turned on. This was usually done in 10-15 and less often in 20-25 pairings of the gas with the light. If no reflex was formed after 25 pairings, the diketene concentration studied was considered to be inactive.

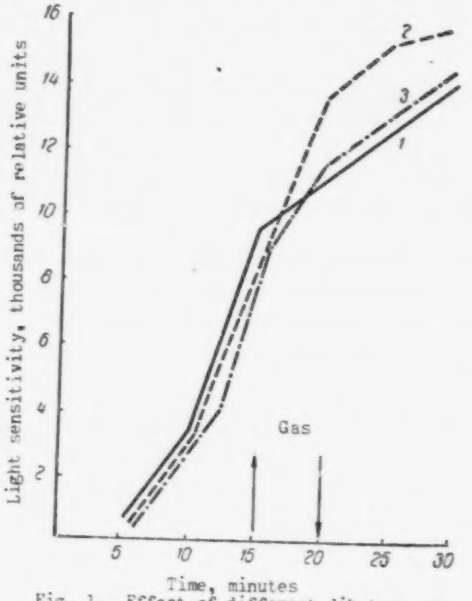

Fig. 1. Effect of different diketene concentrations on the light sensitivity of the eye in subject M. N.
1 - pure air; 2 - 0.021 mg/m^3; 3 - 0.011 mg/m^3.

In order to detect the alpha rhythm desynchronization due to the stimulus studied, we performed a statistical treatment of the data, using the method proposed by V. A. Gofmekler (1964).

Table 2

Reflex Effect of Diketene on the Human Organism.

Subject	Concentration, mg/m³					
	Olfactory Perception		Light Sensitivity of the Eye		Electrical Activity of the Brain	
	Threshold	Sub-Threshold	Threshold	Sub-Threshold	Threshold	Sub-Threshold
Z. I.	0,019	0,011	0,011	0,009		
K. N.	0,019	0,011	0,011	0,009		
D. V.	0,019	0,011	0,02	0,011		
M. N.	0,019	0,011	0,021	0,011	0,01	0,007
A. B.	0,03	0,019			0,019	0,01
S. R.	0,04	0,03			0,019	0,01
K. Ye.	0,04	0,03			0,019	0,01

Results of the study of three diketene concentrations (0.019, 0.01 and 0.007 mg/m^3) are listed in Table 2 and compared with data obtained by studying the threshold of smell and influence of diketene on the light sensitivity of the eye.

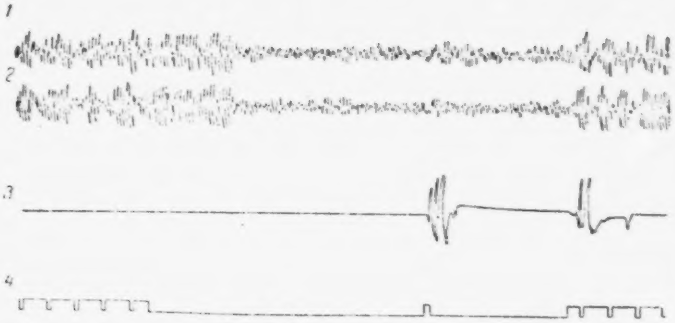

Fig. 2. Conditioned electrocortical reflex on the 17th pairing in subject M. N. during inhalation of diketene in a concentration of 0.01 mg/m^3.

1 - left frontal-and-occipital lead; 2 - right frontal-and-occipital lead; 3 - indication by the subject that the light was turned on; 4 - indication by instrument giving time of action of conditioned and unconditioned stimuli.

It is evident from Table 2 that in three out of the four subjects, the conditioned reflex was developed at a concentration of 0.019 mg/m^3; a concentration of 0.01 mg/m^3 proved to be inactive. In subject M. N. it was possible to develop the conditioned reflex to a lower diketene concentration, 0.01 mg/m^3 (Fig. 2), and the subthreshold concentration was 0.007 mg/m^3.

On the basis of the data of the most sensitive electroencephalographic method, we recommend the highest single maximum permissible concentration of diketene in atmospheric air at a level of 0.007 mg/m^3.

We studied the air pollution around a diketene plant of small capacity. The process of diketene production consists in the pyrolysis of acetone with the formation of a gaseous ketene mixture containing ketene, the absorption of ketene by acetone, and polymerization of ketene into diketene. The air becomes polluted through leaks in the traps, leaks of gases through flanged joints, during repairs of the equipment, and also as a result of exhaust gases which, according to the data of the chemical plants, carry with them out into the air: 23.3 acetone, 2.25 ketene, 12.7 carbon monoxide, 25.7 methane, and 6 kg/hr ethylene. The pollutants are discharged at a height of 20-25 m above ground level through ten stacks.

In May-June 1966, 244 single air samples were taken including 81 for diketene, 82 for ketene, and 81 for acetone. In order to determine diketene and ketene separately, we used the method proposed by the above-mentioned authors (N. V. Stepanyuk et al.), involving parallel collection of two samples in different absorbing solutions - water and sulfuric acid. The diketene content is determined in the first sample, and the combined content of diketene and ketene in the second. The ketene content is found from the difference.

Results of analysis of the samples are given in Table 3.

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Table 3

Concentration of Atmospheric Pollutants Around a Diketene Plant.

Distance from source of discharge, m	Diketene						Ketene					Acetone					
	Number of Samples	Of these, above the sensitivity of the method	Concentration, mg/m^3			Percent of samples above the maximum permissible concentration	Number of Samples	Of these, above the sensitivity of the method	Concentration, mg/m^3			Number of Samples	Of these, above the sensitivity of the method	Concentration, mg/m^3			Percent of samples above the maximum permissible concentration
			Maximum	Minimum	Average				Maximum	Minimum	Average			Maximum	Minimum	Average	
100	28	21	0,022	0,002	0,009	47,6	30	28	0,024	0,001	0,005	26	26	0,90	0,11	0,39	46,2
200	26	20	0,011	0,001	0,006	35,0	26	22	0,010	0,001	0,033	28	27	0,56	0,11	0,28	33,3
300	27	14	0,004	0,001	0,002	—	26	21	0,008	0,001	0,003	27	19	0,33	0,11	0,17	—

The data of this table show that at a distance of 200 m from the source of discharge, the highest single concentrations of diketene and acetone are 1½ times greater than the permissible concentrations, and at a distance of 300 m, the concentrations are within the range of standard levels.

For ketene, the maximum permissible concentration in atmospheric air has not been established. This as well as the lack of data on the range of propagation of other discharges (carbon monoxide, methane and ethylene) makes it difficult to give recommendations on the width of the sanitary protective zone for the production studied.

In the construction of similar new plants of larger capacities, a better system of exhaust gas purification should be provided for.

Conclusions

1. The threshold of odor perception of diketene in the most sensitive persons is 0.019 mg/m^3, but that of the reflex effect on the light sensitivity of the eye is 0.011 mg/m^3, and that of development of the conditioned electrocortical reflex, 0.01 mg/m^3. By these tests, the subthreshold concentrations are respectively 0.011, 0.009, and 0.007 mg/m^3.

2. The highest single maximum permissible concentration of diketene in atmospheric air may be recommended at the level of 0.00[illegible] mg/m^3.

LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1968 bibliography.

EXPERIMENTAL VALIDATION OF THE MEAN DAILY MAXIMUM PERMISSIBLE CONCENTRATION OF VANADIUM PENTOXIDE IN ATMOSPHERIC AIR

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 10, Izdatel'stvo "Meditsina" Moskva, p. 201-217, (1967).

The pollution of air with vanadium pentoxide aerosol may take place as a result of various technological processes in a number of branches of the national economy.

Of the greatest significance in this respect is the metallurgical industry, which utilizes vanadium in the form of ferrovanadium and vanadium pentoxide as an additive to the charge used in the production of alloy steels. Because of the relatively low melting point of vanadium pentoxide (675°C), vapors of the latter can mix with air and on condensing, form an aerosol with particles as small as 2 μ (I. V. Roshchin, 1952, 1963). It is evident that these processes can be associated not only with pollution of the air of plant buildings but also with the escape of vanadium pentoxide aerosol into the atmosphere along with smoke discharges.

Moreover, the contamination of atmospheric air with vanadium pentoxide aerosol may also be due to smoke discharges of various furnace and other systems utilizing bituminous coal as fuel. Low grades of the latter are characterized by a high ash content. However, the ash of bituminous and brown coals, as indicated by the data of V. A. Nazarenko (1937), contains vanadium pentoxide in amounts from 0.44×10^{-2} to 9.3×10^{-2}%.

Because of the presence of vanadium pentoxide in the ash of petroleum, particularly high-sulfur petroleum, in which it is present in amounts from 5 to 17% (I. V. Roshchin, 1962) and also in certain heavy petroleum distillation products, (0.5 to 40% vanadium pentoxide in the ash of mazuts) (Yu. P. Pal'tsev, 1964), air pollution may also be due to smoke discharges from the corresponding heating units and to the exhaust gases of diesel engines (Morette, 1959).

Thus, the development of the metallurgical industry, construction of large-capacity thermal power plants, increase in the number of intracity buses with diesel engines, etc., constitute very probable sources of pollution of atmospheric air with vanadium pentoxide.

This fact was mentioned by a number of researchers in recent years. Thus, Cholak et al. (1952), observed up to 0.2 $\mu g/m^3$ of vanadium in the atmospheric air of the city of Cincinnati and its environs. Morette (1959) found from 11 to 1922 μg of vanadium per g of street dust in Paris. Dohan (1961) found vanadium concentrations from 0.2 to 1.2 $\mu g/m^3$ in the atmospheric air of five American cities. According to the data of our studies, conducted in 1963-1964, around major metallurgical plants, vanadium pentoxide in atmospheric air was observed in 87% of all the samples in the following concentrations:

at a distance of 150 m from the source of discharges, 1.49 ± 0.25 $\mu g/m^3$
at a distance of 500 m from the source of discharges, 0.47 ± 0.09 $\mu g/m^3$
at a distance of 1000 m from the source of discharges, 1.35 ± 0.39 $\mu g/m^3$
at a distance of 1500 m from the source of discharges, 0.98 ± 0.13 $\mu g/m^3$

In 11% of positive samples, the observed vanadium pentoxide concentrations exceeded 2 $\mu g/m^3$.

At the same time, according to literature data, vanadium and particularly its pentoxide affect the organism, causing irritation of the respiratory tract, acute and chronic inflammations of the upper respiratory tract, and changes in the lungs (I. V. Roshchin, 1962; Ye. I. Matantseva, 1960; Kh. G. Gul'ko, 1951; Dutton, 1911, and others). In addition, vanadium and its compounds have a negative effect on the state of the nervous system (Ye. A. Mel'nikova, 1957; K. P. Selyankina, 1961; I. V. Roshchin, 1963; Dutton, 1911, and others), blood picture (Kh. G. Gul'ko, 1951; Symanski, 1939, and others), state of hemodynamics (I. V. Roshchin, 1952), the metabolism (T. A. Prokopenko, 1958; Zewis, 1959, and others). Many authors have also noted changes in various viscera when vanadium and its compounds enter the organism (Ya. B. Reznik, 1954; Molfino, 1939, and others). It is noteworthy that, according to the data of Franke and Moxson (1936,1937), vanadium compounds entering the organism chronically prove to be more toxic than those of arsenic.

Moreover, vanadium compounds in atmospheric air are also significant in the sense that they can promote the oxidation of a part of the sulfur dioxide present in air and its conversion into sulfuric acid aerosol (Tomas, 1959), which, according to the data of K. A. Bushtuyeva (1961), has stronger irritant properties than the initial sulfur dioxide.

As follows from the above, vanadium and its compounds constitute an important ingredient of atmospheric pollutants and should enlist the attention of sanitation services in any evaluation of the atmospheric air of populated areas.

Among the various compounds of vanadium, the most important is the pentoxide, which on the one hand is among the most toxic compounds of vanadium (I. V. Roshchin, 1952, 1963), and on the other hand, because of its great volatility, may escape into the atmosphere in relatively large amounts and more often than the other compounds.

On the basis of the above, and also in view of the recommendations of the Committee on Sanitary Protection of Atmospheric Air, it was decided to undertake a sanitary validation of the mean daily maximum permissible concentration of vanadium pentoxide in atmospheric air.

Accordingly, in the summer of 1964, we conducted the first series of a 70-day round-the-clock continuous exposure of animals to vanadium pentoxide. The experiment involved the use of 33 white male rats with an initial weight of 110-130 g, which were divided into three groups. The rats of group I were exposed to vanadium pentoxide in a concentration 0.027 ± 0.002 mg/m^3 of air, a value 3.7 times lower than the maximum permissible concentration of vanadium pentoxide established for the air of industrial buildings (0.1 mg/m^3). The rats of group II were subjected to vanadium pentoxide in a concentration of 0.002 - 0.00013 mg/m^3 of air, i. e., a concentration close to those which we found near metallurgical plants which do not utilize vanadium for any special purposes. The rats of group III served as the control.

The exposure was carried out in three chambers, each with a 100-l capacity. The air was supplied to the chambers at a rate of 30-33 l/min, which provides for an adequate air exchange (V. A. Popov, 1964).

Considering that vanadium pentoxide is most frequently found in air in a condensed state, to generate the condensation aerosols of vanadium pentoxide we used a tubular furnace containing a dish with molten vanadium pentoxide. To ensure a constant rate of vaporization of vanadium pentoxide, the temperature in the furnace was stabilized at 800°C by means of a specially designed temperature control device. Finely dispersed vanadium pentoxide aerosol was fed into the chambers by passing air through the furnace at a rate of 2.5-3 l/min. The air supply unit with the aerosol generator was separated from the chamber containing the animals to exclude the effect of noise on the latter.

To determine the vanadium pentoxide concentrations in the chambers, the air samples were collected on AFA-KhA-18 filters in the course of the entire period of exposure. The filters were consumed in a mixture of concentrated nitric and sulfuric acids. The chemical determination of vanadium pentoxide was carried out by M. V. Nifontova's method (1960) using an FEK-M photoelectrocolorimeter. The data obtained were treated statistically.

To evaluate the chronic effect of vanadium pentoxide on the animal organism during the exposure and to get an idea of the condition of the animal organism before the exposure and during the recovery period, we utilized the following indicators: general condition and weight of the animals, motor chronaxy of antagonist muscles, activity of blood cholinesterase, total protein and protein fractions of blood serum, oxyhemoglobin content of venous blood, and fluorescent* picture of blood leucocytes. In addition, after the completion of exposure, the absorption of oxygen by ground-up liver tissue was determined in Warburg's apparatus, and some viscera of the experimental animals were removed for histopathomorphological examinations. The statistical significance of the results of the studies was determined in the course of the observation. During the exposure, all the animals (both experimental and control groups) gained weight satisfactorily and were active.

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*Editor's note: For the Russian use of the terms "luminescent" and "luminescence" in this paper, we have substituted "fluorescent" and "fluorescence", on the basis of the definitions of these terms.

In view of the fact that at the present time one of the standard tests in the hygienic validation of maximum permissible concentrations of noxious substances in atmospheric air is the determination of the motor chronaxy of antagonist muscles (V. A. Ryazanov, 1961), we made use of this test in our work.

The motor chronaxy of antagonist muscles of the right rear shin of the rats was determined with the aid of an ISE-01 electronic pulse stimulator, always under the same conditions, every 10 days, using the accepted procedure. In the course of the chronic exposure, the motor chronaxy of antagonist muscles in rats of group II did not change relative to the control group of animals (Table 1). In rats of group I, the motor chronaxy during the period of exposure underwent substantial changes which were manifested, on the one hand, in a statistically significant decrease of the chronaxy of extensors by an average of 0.8 msec during the period of exposure, and on the other hand, in an even more pronounced increase of the chronaxy of flexors by an average of 4 msec during that period. More illustrative is the change in the chronaxial ratio of antagonist muscles in this group of rats. As is evident from Table 1, as early as the 20th day of exposure, an inhibitory process developed in the central nervous system of rats of group I; as a result of the altered subordinating influence of the central nervous system on the periphery (Yu. M. Uflyand, 1941), this process manifested itself in an equalization of the chronaxies of antagonist muscles and extensors of the shin. On the 30th day, this process became more serious, and the ratio between the chronaxies of flexors and extensors was reversed. This reversal of the ratio lasted until the end of exposure in animals of group I. After 20 days of the recovery period, the indices of the motor chronaxy of flexors and extensors of the shin and their ratio returned to the original level in the rats of this group.

As is known from literature data and also confirmed in our work by the study of the chronaxy of antagonist muscles, the influence of vanadium pentoxide on the central nervous system served as the basis for using also the method of determination of blood cholinesterase activity in this study. As follows from the work of D. Ye. Al'pern (1958, 1963), a major part in the regulation of blood cholinesterase activity is played by the state of the nervous system, particularly of the cerebral cortex. On this basis, we postulated that the active concentrations of vanadium pentoxide in the inhaled air, which affect the central nervous system, may also influence the activity of blood cholinesterase.

The blood cholinesterase activity was determined by the method of Hestrin

Table 1.

Motor Chronaxy of Antagonist Muscles in Rats During Their Exposure to Vanadium Pentoxide.

	Group of rats	Statistical indices	Period of study (decades)					Period of study (decades)					
			Before exposure		During exposure			During exposure				During recovery period	
			1	2	1	2	3	4	5	6	7	1	2
Chronaxy of flexors in µsec	I	M ± m	31,0 ± 1,73 (o)	34,7 ± 5,24 (o)	35,3 ± 7,80 (o)	45,7 ± 3,29 (b)	74,3 ± 1,34 (c)	78,3 ± 7,69 (b)	74,7 ± 9,94 (a)	91,0 ± 2,51 (c)	88,3 ± 5,37 (c)	38,5 ± 0,50 (a)	32,5 ± 1,50 (o)
	II	M ± m	24,7 ± 3,51 (o)	26,7 ± 4,25 (o)	28,3 ± 4,91 (o)	34,7 ± 7,84 (o)	30,3 ± 4,06 (c)	26,7 ± 3,18 (o)	24,3 ± 3,67 (o)	23,7 ± 3,18 (o)	24,0 ± 3,61 (o)	22,7 ± 3,51 (o)	20,5 ± 1,50 (o)
	III	M ± m	28,0 ± 2,31	32,0 ± 4,00	23,3 ± 2,73	30,0 ± 0,58	32,3 3,67	33,3 ± 0,89	31,0 ± 1,00	30,3 ± 0,34	29,3 ± 1,76	32,5 ± 0,50	29,5 ± 1,50
Chronaxy of extensors in µsec	I	M ± m	50,0 ± 3,21 (o)	55,0 ± 7,55 (o)	39,7 ± 7,06 (o)	45,0 ± 3,79 (o)	44,3 ± 5,55 (o)	41,0 ± 5,13 (o)	27,7 ± 3,85 (a)	33,7 ± 6,33 (o)	31,7 ± 6,20 (o)	34,0 ± 7,00 (o)	51,7 ± 3,51 (o)
	II	M ± m	41,7 ± 4,25 (o)	49,3 ± 5,46 (o)	45,0 ± 7,77 (o)	58,0 ± 11,1 (o)	48,0 ± 6,03 (o)	43,7 ± 5,24 (o)	41,7 ± 8,11 (o)	41,0 ± 3,21 (o)	41,3 ± 3,33 (o)	40,0 ± 8,00 (o)	35,0 ± 4,00
	III	M ± m	43,0 ± 1,73	45,7 ± 3,93	38,0 ± 4,51	45,3 ± 1,67	49,0 ± 3,21	51,3 ± 0,89	49,0 ± 3,79	48,7 ± 1,76	46,0 ± 2,80	48,7 ± 4,51	44,3 ± 1,53
Index of ratio of chronaxy of flexors to that of extensors (x 100)	I	M ± m	63 ± 0,89 (o)	62 ± 1,73 (o)	86 ± 5,00 (b)	108 ± 9,40 (a)	172 ± 19,7 (b)	193 ± 14,5 (c)	270 ± 30,0 (b)	293 ± 40,0 (b)	263 ± 31,8 (b)	118 ± 72,0 (o)	63 ± 1,00 (o)
	II	M ± m	61 ± 2,41 (o)	60 ± 5,13 (o)	64 ± 6,90 (o)	60 ± 3,48 (o)	63 ± 4,06 (o)	63 ± 5,70 (o)	60 ± 4,40 (o)	58 ± 4,20 (o)	59 ± 4,40 (o)	57 ± 3,00 (o)	61 ± 10,0 (o)
	III	M ± m	65 ± 2,31	70 ± 3,53	60 ± 1,86	662 ± 2,70	66 ± 4,10	65 ± 0,55	64 ± 3,18	63 ± 4,50	61 ± 1,53	68 ± 5,50	66 ± 1,00

Note. Degree of significance: a - 95%; b - 99% c - 99.9% 0 - not significant

(1951) and evaluated in gammas of acetylcholine decomposed by blood cholinesterase in 1 minute. The results of the studies are given in Table 2.

Table 2.

Activity of Blood Cholinesterase in Rats
(in gammas of acetylcholine decomposed by 1 ml of blood per minute).

Period	Statistical indices	Group		
		I	II	III III (control)
Before exposure	M ± m	120,0±7,20 (o)*	121,7±7,45 (o)	127,7±3,13
Exposure	M ± m	98,1±4,95 (a)	119,4±3,33 (o)	119,1±5,53
Recovery period	M ± m	81,0±4,62 (a)	104,8±3,95 (o)	100,0±6,77

*Degree of significance: a - 95%; o - not significant.

As was shown by the statistical treatment of the results, a significant decrease of blood cholinesterase activity occurred only in animals of group I during the period of exposure. This decrease continued to be observed during the entire recovery period. In animals of groups II and III (control), no significant change in cholinesterase activity was observed in any of the periods. In addition to using the above methods, we also determined the content of total protein and protein fractions in the blood serum.

It is known from the literature data that the introduction of vanadium into the animal organism causes a decrease of protein synthesis (Snyder and Cornatzer, 1958), and also increases the content of gamma globulins in the blood serum (Joya, 1958). The total protein of the blood serum was determined by Kjeldahl's micromethod, and its quantity was expressed in gram-percent. The results of the study are given in Table 3.

The studies showed that in animals of group I, the amount of protein of the blood serum after 40 days of exposure remained practically the same as before the exposure, whereas in animals of groups II and III (control) it increased; this is due to the growth of the organism and is normal. S. D. Balakhovskiy and I. S. Balakhovskiy (1953) give the average contents of total protein in the blood of rats as ranging from 4.8 g% in young ones to 7.2 g% in adults. Thus, under the influence of vanadium pentoxide, an inhibition of the synthesis of blood serum proteins took place in rats of group I. The difference between the content of the latter in the blood serum of rats of group I as compared with group III (control) was found to be statistically significant.

Table 3.

Change in the Amount of Total Protein in the Blood of White Rats During Their Exposure to Vanadium Pentoxide.

Period	Statistical indices	Group		
		I	II	III (control)
Before Exposure	M ± m	6,00 ± 0,15 (o)*	4,80 ± 0,73 (o)	5,75 ± 0,00
Exposure	M ± m	6,17 ± 0,03 (b)	6,67 ± 0,53 (o)	6,65 ± 0,05

*Degree of significance: b - 99%; o - not significant.

The protein fractions of the blood serum were determined by paper electrophoresis. The phoregrams were analyzed by elution of the dye followed by colorimetry on FEK-M. The results are shown in Table 4.

No reliable changes were observed during the entire period of exposure in the index of the ratio of albumins to globulins.

The content of the protein fractions of the blood serum in rats of group II did not differ appreciably from that of rats of the control group. In animals of group I, a statistically significant increase of gamma globulins in the protein fractions of the blood serum occurred on the 40th day of exposure. At the same time, there was a certain significant decrease of beta globulins.

The method of fluorescent microscopy of leucocytes with vital staining of the specimens has already been applied in practice to the validation of maximum permissible concentrations of atmospheric pollutants (M. I. Gusev, K. N. Chelikanov, 1963). We used the method of preparation of specimens with supravital staining with acridine orange diluted in the proportion of 1:20,000 (Ye. B. Zakrzhevskiy and L. G. Vasil'yeva, 1963). The specimens thus prepared do not lose their vital advantages while at the same time permitting one to carry out the microscopy during a much longer time interval (one month according to our observations).

The microscopy of the specimens was carried out by means of an MUF-3M microscope with an OI-23M illuminator in violet light under immersion. The normal and altered forms of leucocytes were distinguished by the nature of the lighting of their nuclei.

As we know from literature data (Ye. B. Zakrzhevskiy and L. G. Vasil'yeva, 1963), the fluorescence of leucocytes depends on the degree of polymerization of the fluorochrome molecules, which in turn depends on the state of the DNA

Table 4.

Content of Protein Fractions in the Blood Serum of Rats (in %).

Period of observation	Protein fraction	Statistical indices	Group		
			I	II	III (control)
Before exposure	Albumins	M ± m	41,9 ± 1,10 (o)	41,8 ± 1,41 (o)	41,0 ± 0,48
	Alpha globulins	M ± m	24,4 ± 2,12 (o)	23,3 ± 1,09 (o)	24,9 ± 0,14
	Beta globulins	M ± m	21,0 ± 1,21 (o)	22,4 ± 0,84 (o)	20,9 ± 0,42
	Gamma globulins	M ± m	12,5 ± 1,16 (o)	12,6 ± 1,56 (o)	1,31 ± 0,08
	Albumin-globulin ratio	M ± m	0,72 ± 0,03 (o)	0,72 ± 0,04 (o)	0,70 ± 0,02
Exposure	Albumins	M ± m	39,8 ± 2,39 (o)	40,0 ± 0,67 (o)	41,5 ± 0,53
	Alpha globulins	M ± m	23,5 ± 1,02 (o)	25,2 ± 0,47 (o)	23,1 ± 2,51
	Beta globulins	M ± m	18,4 ± 0,13 (a)	22,4 ± 0,99 (o)	23,7 ± 3,28
	Gamma globulins	M ± m	18,5 ± 1,49 (a)	12,4 ± 1,56 (o)	11,7 ± 0,24
	Albumin-globulin ratio	M ± m	0,67 ± 0,07 (o)	0,67 ± 0,03 (o)	0,71 ± 0,01
Recovery period	Albumins	M ± m	37,4 ± 1,28 (o)	39,4 ± 5,04 (o)	36,7 ± 1,40
	Alpha globulins	M ± m	22,9 ± 3,21 (o)	22,9 ± 2,88 (o)	26,7 ± 1,85
	Beta globulins	M ± m	24,7 ± 4,20 (o)	24,1 ± 2,94 (o)	22,9 ± 0,66
	Gamma globulins	M ± m	15,1 ± 0,41 (o)	13,7 ± 0,78 (o)	13,7 ± 2,60
	Albumin-globulin ratio	M ± m	0,60 ± 0,04 (o)	0,66 ± 0,01 (o)	0,59 ± 0,0[illegible]

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*Degree of significance: a - 95%; o - not significant.

and RNA molecules in the protoplasm and cell nucleus. Of great importance are also the degree of polymerization of the nucleic acids themselves, the nature of their complex bond with acridine orange, and the chemical structure of the nucleic acids. Nondenatured nucleic acids have a helical structure and produce a green fluorescence, whereas denatured ones are in the form of single spirals and produce a red fluorescence.

At the start of the process of rearrangement of DNA in the cell nucleus, yellow fluorescence appears first instead of the normal green, and later it turns orange and then red. When the blood picture is normal, only isolated leucocytes with altered fluorescence are found, which may be due to their natural death.

The results of our studies are given in Table 5.

Table 5.

Number of Leucocytes with Altered Fluorescence (in percent).

Period of observation	Statistical indices	Group		
		I	II	III (control)
Before exposure	M ± m	1,25 ± 0,70 (o)	1,25 ± 0,70 (o)	1,25 ± 0,70
Exposure: After				
20 days	M ± m	5,75 ± 0,75 (b)	2,00 ± 0,82 (o)	2,25 ± 0,25
45 days	M ± m	13,75 ± 3,84 (a)	1,75 ± 0,75 (o)	1,25 ± 0,25
70 days	M ± m	20,25 ± 5,40 (a)	1,25 ± 0,25 (o)	1,25 ± 0,25
Recovery period	M ± m	1,25 ± 0,63 (o)	1,00 ± 0,58 (o)	1,50 ± 1,50

Note. Degree of significance: a - 95%; b - 99%; c - 99.9%;
o - not significant.

As is evident from Table 5, no appreciable changes in the fluorescent picture of the blood occurred in rats of groups II and III (control) over the course of the entire experiment. In rats of group I, the number of leucocytes with yellow fluorescence of the nuclei increased, and also, cells with orange and red nuclei appeared. Statistical treatment of the results showed that a significant increase in the number of leucocytes with altered fluorescence was observed in rats of group I as early as the 20th day of exposure and grew until the end of latter. After 20 days of the recovery period, the fluorescence of the blood leucocytes of this group of animals returned to the original level.

Some authors contend that vanadium can affect the oxidation-reduction processes in the organism (Trummert and Boehm, 1957; Snyder and Cornatzer, 1958; Mitchell, 1953). At vanadium pentoxide concentrations in the air of industrial buildings in excess of the maximum permissible values, the workers show hyperventilation of the lungs (Ye. I. Matantseva, 1960). In an experiment on animals, vanadium caused an increase in oxygen consumption in Mitropol'skiy's apparatus (K. P. Selyankina, 1961). In this connection, we considered it expedient to use the oxyhemometry method in our experiment. The blood was taken from the caudal veins by chopping off the tip of the tail. The content of oxyhemoglobin in percent was measured on a model 0-57 combination oxyhemometer. The results of the analyses are given in Table 6.

Table 6.

Oxyhemoglobin Content of Venous Blood of Rats (percent).

Period of observation	Statistical indices	Group		
		I	II	III (control)
Before exposure	M ± m	60,0 ± 1,8 (o)*	60,0 ± 1,8 (o)	60,0 ± 1,8
Exposure	M ± m	51,5 ± 2,9 (b)	59,6 ± 2,1 (o)	61,2 ± 1,3
Recovery period	M ± m	63,0 ± 1,0 (o)	62,0 ± 2,0 (o)	59,0 ± 2,0

*Degree of significance: b - 99%; o - not significant.

Because of a certain scatter of the data, the average oxyhemoglobin content in the blood of rats before exposure was obtained from the results of studies of all the groups of rats. Changes in the oxyhemoglobin content of the blood were found to be statistically significant only in rats of group I. Such changes were not observed in animals of groups II and III. After 20 days of the recovery period, the oxyhemoglobin content in rats of group I returned to the original value.

A definite idea of the participation of vanadium in the oxidation-reduction processes in the organism is also given by the experiment of F. and M. Berngeym (A. O. Voynar, 1953). The authors showed that the addition of vanadium compounds (metavanadate, vanadium acetate) in amounts corresponding to 10-20 g of vanadium increases the respiration of finely ground liver of higher animals, raising the oxygen requirement by a factor of 10. In this connection, it was of interest to determine the extent to which the exposure of animals to vanadium pentoxide in our experiment affected the tissue respiration of the liver of the experimental animals. Accordingly, after the completion of exposure, the liver was removed from 11 animals of groups I, II and III, killed by decapitation. The respiration of the ground liver tissue was studied in Warburg's apparatus.* The results of the studies are given in Table 7.

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*The determination was made by V. G. Podterger', a graduate student in the biochemistry department, with the participation of the author.

Table 7.

Consumption of Oxygen by 500 mg of Ground-up Liver in Warburg's Apparatus in the Course of 20 Minutes (in microliters).

Statistical indices	Group		
	I	II	III Control
$M \pm m$	263.3 ± 14.9 (a) 124%	$222{,}0 \pm 11.9$(o) 104%	$212{,}3 \pm 4{,}26$ 100%

Note. Degree of significance: a - 95%; o - not significant.

As is evident from Table 7, in rats of group I, the ground liver tissue consumed 24% more oxygen than that of rats of group III (control). Attention is drawn to the fact that the degree of increase in oxygen consumption by the liver in our experiment was considerably more than in the Bernheims' experiment, apparently because of the characteristics of the experiment: the latter authors added the vanadium compounds directly to the ground liver tissue in vitro, whereas in our experiment the animals received vanadium pentoxide while alive by inhalation. The consumption of oxygen by the liver of rats of group II reliably showed no difference from that of the control group.

Histological examination of certain viscera of the rats* revealed pathological changes only in rats of group I. The lungs showed an acute hyperemia in the vessels, focal blood extravasations, signs of panbronchitis; the liver, an acute hyperemia in the central veins, fine isolated blood etravasations around them, isolated infiltrates between the liver lobes, and signs of granular degeneration of the liver cells. The kidneys showed a pronounced granular degeneration of the epithelium of the convuluted tubules, attaining necrosis in some areas, and the myocardium showed an acute hyeremia of the vessels with focal blood extravasation around them.

Thus, the studies showed that a vanadium pentoxide concentration of 0.027 mg/m^3 of air in chronic round-the-clock exposure of animals for 70 days causes functional and morphological changes in the organism of the experimental animals, whereas a concentration of 0.002 mg/m^3 of this substance fails to cause such changes under the same conditions.

Considering that the vanadium pentoxide concentrations that we studied were taken with a large gap, we conducted an additional, second series of experiments for the purpose of studying an intermediate concentration that would be closer to the inactive concentration.

The experiment was set up on 20 young white male rats. The latter were divided into two groups. The first experimental group was exposed to vanadium pentoxide in a concentration of 0.006 ± 0.00056 mg/m^3 of air, and group II was the control. The exposure was conducted in 1964-65 continuously for 40 days.

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* The histopathological examinations were performed by Candidate of Medical Sciences G. P. Zlatin.

Table 8.

Number of Leucocytes with Altered Fluorescence of Nuclei (in percent) (second series).

Period of observation	Statistical indices	Group	
		Experimental	Control
Before exposure	M ± m	0,67 ± 0,33 (o)	0,67 ± 0,33
Exposure:			
After 15 days	M ± m	1,50 ± 0,56 (o)	0,60 ± 0,40
After 30 days	M ± m	3,17 ± 1,25 (o)	1,67 ± 0,42
After 40 days			
After starvation	M ± m	4,83 ± 0,79 (b)	1,00 ± 0,37

Note. Degree of significance: b - 99%; o - not significant.

In order to expose the shifts in the functional state of the experimental animals, tests were selected which made it possible to reveal the earliest shifts in the first series of experiments, namely, a study of the chronaxy of antagonist muscles and of the fluorescent picture of blood leucocytes. In addition, the general state and weight of the animals were followed. The motor chronaxy of antagonist muscles was determined in four rats of each group. No reliable differences in the chronaxy of antagonist muscles in rats of the experimental group were found in the course of the first month of exposure as compared with the control. However, at the end of the first month of exposure, slight reliable shifts occurred in the chronaxial ratio of the flexors and extensors.

The fluorescent picture of the blood leucocytes was studied in 6 rats of each group (Table 8).

During the first month of exposure, there were no reliable differences in the fluorescent picture of the blood in rats of the experimental group as compared with the control.

Considering that the initial shifts in the organism of the animals are better exposed under a functional load, on the 6th week of exposure we used a 3.5-day starvation of the animals, which during that time received only water. Studies carried out after the 3.5-day starvation of the animals revealed significant shifts in the functional state of animals of the experimental group. Thus a statistically significant change occurred in the chronaxial ratio of flexors and extensors of the shin in rats of the experimental group. A reliable fivefold increase in the number of leucocytes with altered fluorescence as compared with the control took place in the fluorescent picture of the blood leucocytes in rats of the experimental group.

Conclusions

1. A vanadium pentoxide concentration of 0.027 mg/m^3 in chronic round-the-clock exposure of the animals for 70 days causes substantial changes in the functional state of the organism of the experimental animals, and morphological changes in certain viscera.

2. A vanadium pentoxide concentration of 0.006 mg/m^3 in a continuous 40-day exposure also caused slight changes in the functional state of the organism of the animals. After the functional load of starvation was used, these changes became considerably more serious.

3. A vanadium pentoxide concentration of 0.002 mg/m^3 was found to be inactive.

4. On the basis of the above studies, the concentration of 0.002 mg/m^3 is recommended as the mean daily maximum permissible concentration of vanadium pentoxide in atmospheric air.

LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1967 bibliography.

DETERMINATION OF THE THRESHOLD OF THE REFLEX EFFECT OF CHLOROPRENE BY THE METHOD OF ELECTROENCEPHALOGRAPHY

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 11, Izdatel'stvo "Meditsina" Moskva, p. 171-179, (1968).

An electroencephalographic determination of the threshold of the reflex effect of chloroprene was undertaken at the suggestion of the Section on Sanitary Protection of Atmospheric air in order to define more accurately the maximum permissible highest single concentration of chloroprene in atmospheric air adopted in the USSR (0.25 mg/m^3), which we proposed on the basis of experimental studies aimed at determining the olfactory threshold of chloroprene and the threshold of its influence on the light sensitivity of the eye. According to the data of these studies, the threshold of olfactory perception of chloroprene coincided with the threshold of its reflex effect on the functional state of the visual system as determined by the dark adaptation method, and for the most sensitive persons amounted to 0.4 mg/m^3 for a maximum inactive concentration of 0.25 mg/m^3 (A. V. Mnatsakanyan, 1961).

The unsaturated chloro-substituted hydrocarbon chloroprene, discharged into atmospheric air in the course of production of chloroprene synthetic rubber, has a strong odor and is an irritant. In addition, it has a marked resorptive effect on a number of organs and systems, with a particular influence on the central nervous system.

We recorded the electrical activity of the subjects' brain on an 8-channel German encephalograph with ink recording. The curve of spontaneous activity of the cortex of the large hemispheres was recorded on six channels by the bipolar method. In selecting the leads, we were guided by two considerations: on the one hand, as we know, the alpha rhythm is best and most commonly recorded in the occipital areas of the brain, and on the other hand, the olfactory centers are located in the frontal-and-temporal regions, and as indicated above, chloroprene has a considerable olfactory effect.

In our studies, we selected persons with a distinct and constant alpha rhythm and a normal olfactory function. During the period of this study, they were placed in an insulated and shielded room that was completely darkened. In front of the subject was a cylinder through which pure air was continually supplied at a rate of 30 l/min. At a predetermined time, the gas studied, delivered through a system of stopcocks from a dispenser, was added in a certain concentration to the pure air. To study the influence of chloroprene on the electrical activity of the brain, we used the method of formation of the conditioned electrocortical reflex to the inhalation of chloroprene; to this end, the light was paired with the subthreshold and threshold odor concentrations of chloroprene. The isolated action of chloroprene lasted 15 seconds, and during the following 5 seconds was paired with the action of continuous light.

In order to select persons with a distinct and constant alpha rhythm, a repeat recording of the electroencephalograms was made with 28 volunteers consisting of scientific workers and staff of the institute's laboratory (19 women and 9 men). In 8 persons out of 28 (including 4 men out of 9), no alpha rhythm was observed. In the remaining ones, however (71%), the alpha rhythm appeared as either single waves or irregular groups of waves against a background of beta rhythm, or as a diffuse jumble of rhythms (8 persons), or else it was well-defined and dominant (12 persons, including 1 man). Among the latter 12 persons, the alpha rhythm was constant only in four, one of whom (the man) showed almost no response to the light. In addition to three women with a marked and constant alpha rhythm (Kh. I., Kh. A., and Ch. A.), we selected another five (V. O., Sh. A., T. A., O. T., and V. K.) with a dominant alpha rhythm giving different degrees of modulation. In order to develop a constant alpha rhythm, a "functional compensator" was used, constructed and installed by the engineer B. N. Balashev. It should be noted that by holding the subject's attention, this instrument helped in keeping him awake. However, by preventing the occurrence of a somnolent state, the subject's concentration on the indicator of the galvanometer, which had to be merely reset at zero if it had deflected, did not yield perceptible results from the standpoint of development of a constant alpha rhythm. It is our impression that one could achieve a larger alpha rhythm by deflecting the galvanometer indicator a certain number of divisions and requiring a certain reply from the subject (after a preliminary understanding). Not having had this opportunity, we resorted to a different method: the subject was asked to count the number of times that the light was turned on, and after it was disconnected, to "limber up," i. e., make certain light movements with his arm (chiefly in the radiocarpal and partly in the elbow joint), which varied with the sequence number of the light. These assignments were changed periodically. Our experience shows that for the majority of subjects, such "limbering up" gives a positive result in terms of the development of a fairly constant alpha rhythm and reduces its damping to a minimum.

During November-December 1965 and January-March 1966, we recorded about 200 encephalograms. We thus tested chloroprene concentrations of 0.08, 0.1, 0.2 and 0.3 mg/m^3, of which the first three proved imperceptible in terms of smell for all of the subjects. The concentration of 0.3 mg/m^3 was perceptible in terms of smell for three out of five subjects.

Analysis of the material obtained demonstrated that the 0.3 mg/m^3 chloroprene concentration had a reflex effect on the electrical activity of the brain in all five subjects studied. In 18 experiments out of 21, a conditioned electrocortical reflex was developed to the pairing of this chloroprene concentration with light. The electrocortical reflex was manifested as a desynchronization of the alpha rhythm before the light was turned on the first time on the 5th-20th pairing, on different days and in different persons. For the most part, once it appeared, the desynchronization of the alpha rhythm was repeated many times in a row (in 5-10 pairings), following which a decrease of the reflex effect was observed as a result of adaptation of the nerve cells. However, even on the 30th pairing of the gas with light, the conditioned reflex did not disappear completely.

As was noted above, for three out of five persons, the 0.3 mg/m^3 chloroprene concentration was found to be perceptible in odor; the subjects described it as "offensive" or "stale" and perceived it for the most part in the first two to three inhalations. In these persons, the 0.3 mg/m^3 chloroprene concentration affected the biocurrents of the brain during isolated inhalation of the gas; the effect was manifested as a complete depression of the alpha rhythm or decrease of its amplitude during inhalation of the gas in the course of the entire period (15 seconds) or of a part of it. It follows from the above that while being imperceptible in odor for two of the subjects, the 0.3 mg/m^3 chloroprene concentration had a reflex effect on the electrical activity of the cerebral cortex (Fig. 1 and 2).

The 0.2 mg/m^3 chloroprene concentration paired with light for the same five persons was tested 21 times; in two of them (Kh. A. and Sh. A.) we were unable to develop a conditioned electrocortical reflex.

Thus, for these subjects, the threshold of olfactory perception of chloroprene coincided with the threshold of the reflex effect on the electrical activity of the brain and amounted to 0.3 mg/m^3. For the remaining three persons, the chloroprene concentration of imperceptible odor (0.2 mg/m^3) had a reflex effect when paired with light in the absence of a direct influence on the electrical activity of the brain in the case of isolated use of the gas. In subject Kh. I., the supply of chloroprene in 0.2mg/m^3 concentration paired with light, in addition to developing a conditioned reflex, caused a change in the character of the brain's biocurrents in certain cases. In subject V. L., the reflex effect of chloroprene in 0.2 mg/m^3 concentration was manifested in the development of a conditioned reflex to the pairing of this concentration with light on the 6th-9th pairing. In one case, a brief change in the character of the brain's biocurrent was noted in this subject after the conditioned reflex had appeared. In subject Ch. A., a conditioned reflex was successfully developed in only one experiment out of six, on the 5th pairing of chloroprene in 0.2 mg/m^3 concentration with light. Thus, the subthreshold chloroprene concentration for smell (0.2 mg/m^3) had a reflex effect on the electrical activity of the brain in three out of five subjects (Figs. 3 and 4).

The 0.08-0.1 mg/m^3 chloroprene concentration was tested on five persons in 32 experiments; in no case was an electrocortical reflex developed.

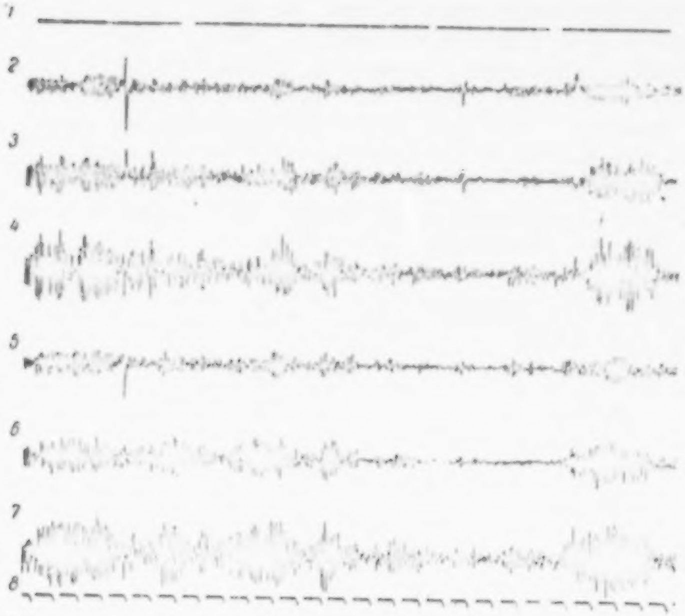

Fig. 1. Change in the electrical activity of the brain in subject Sh. A. during the action of chloroprene in a concentration of 0.3 mg/m^3 (pairing of gas with light).
1 - marks indicating the connection of chloroprene, connection of light, and disconnection of chloroprene and light; 2-7 - recording of biocurrents from successive leads; 2 - frontal-and-parietal; 3 - occipital-and-parietal and 4 - frontal-and-occipital left hemisphere; 5-7 - from the same leads of the right hemisphere; 8 - time mark.

In addition to the material cited above for five persons, we carried out similar studies on another three persons. In three out of five experiments with pairings of the 0.2 mg/m^3 chloroprene concentration with light, a conditioned electrocortical reflex was developed, whereas it was not developed in any of 11 experiments with pairings of chloroprene in concentrations of 0.08-0.1 mg/m^3 with light.

Summing up all of the available material, we came to the conclusion that the 0.2 mg/m^3 chloroprene concentration, the odor of which is imperceptible, has a reflex effect on the electrical activity of the brain in the most sensitive persons. This test showed a chloroprene concentration of 0.08-0.1 mg/m^3 to be inactive, and on this basis we propose that the highest single maximum permissible concentration of chloroprene in atmospheric air be lowered from 0.25 to 0.1 mg/m^3. At the same time, we consider it possible to set the mean daily maximum permissible concentration of chloroprene in atmospheric air, earlier established simultaneously with the highest single

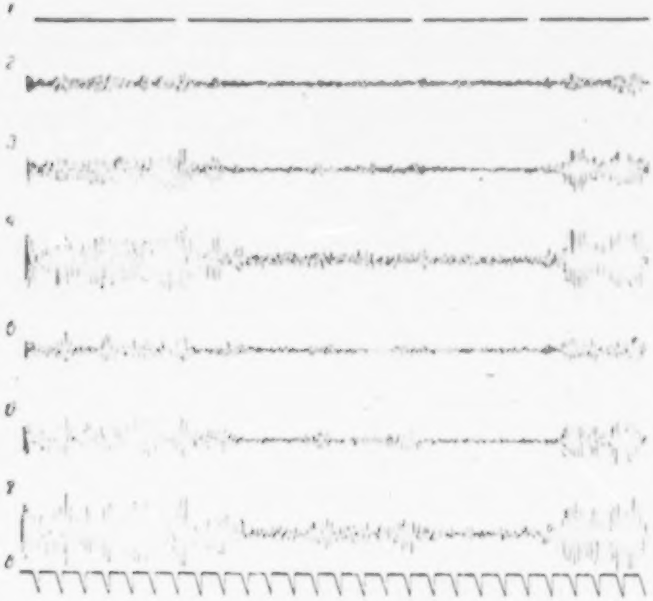

Fig. 2. Change in the electrical activity of the brain in subject Sh. A. during the action of chloroprene in a concentration of 0.3 mg/m^3 (15th pairing of gas with light). Notation same as in Fig. 1.

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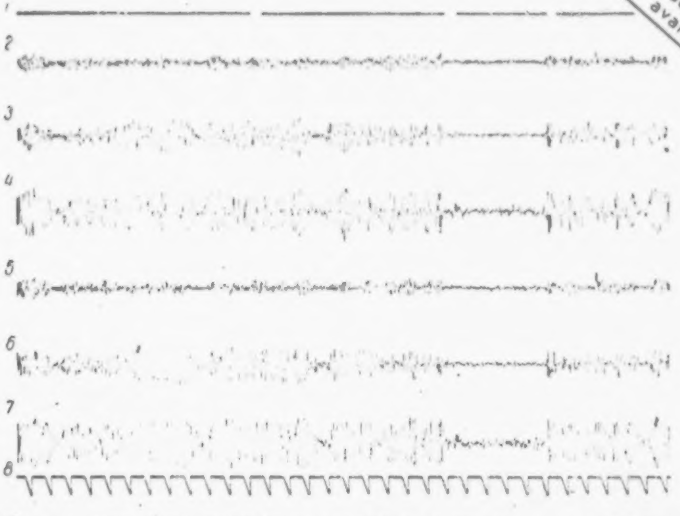

Fig. 3. Electrical activity of the brain in subject Kh. I. during the action of chloroprene in a concentration of 0.2 mg/m^3 (10th pairing of gas with light). Notation same as in Fig. 1.

concentration at 0.08 mg/m^3, equal to the same level (0.1 mg/m^3), i. e., at one-third of the highest single value. Later, having used a whole series of tests in a round-the-clock 60-day exposure of rats to chloroprene, we found that a chloroprene concentration of 0.088 mg/m^3 may be considered inactive for a minimum active concentration of 0.22 mg/m^3 (A. V. Mnatsakanyan, 1964).

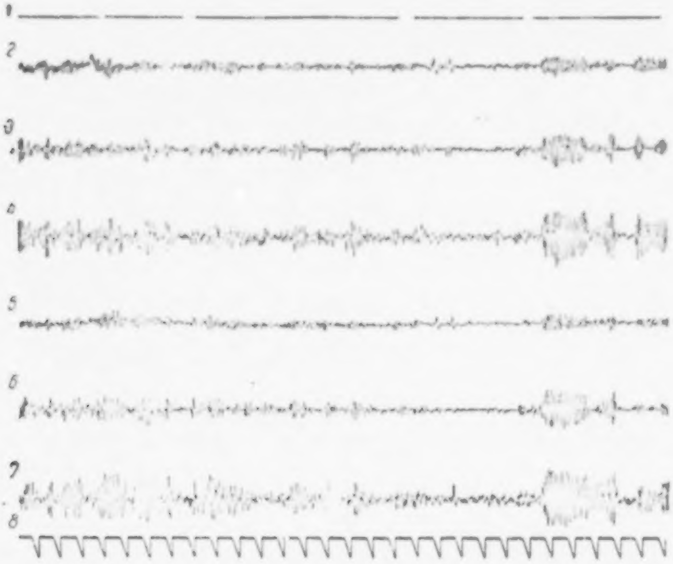

Fig. 4. Change in the electrical activity of the brain in subject Kh. I. during the action of chloroprene in a concentration of 0.2 mg/m^3 (14th pairing of gas with light). Notation same as in Fig. 1.

Conclusions

1. The threshold of the reflex effect of chloroprene, determined by the electroencephalographic method in the most sensitive persons, is 0.2 mg/m^3, i. e., below the olfactory threshold and threshold of influence of chloroprene on the dark adaptation of the eye.

2. The material obtained serves as the basis for a review of the highest single maximum permissible concentration of chloroprene in atmospheric air in the direction of a decrease from 0.25 to 0.1 mg/m^3.

3. At the same time, we consider it possible to set the mean daily maximum permissible concentration of chloroprene in atmospheric air equal to the same level (0.1 mg/m^3). This change is permissible, since in a round-the-clock 60-day experiment, a chloroprene concentration of 0.088 mg/m^3 was found to be inactive for white rats in a series of tests.

LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1968 bibliography.

ON THE TOXICITY OF MICROCONCENTRATIONS OF ETHYLENE OXIDE

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 10, Izdatel'stvo "Meditsina" Moskva, p. 54-64, (1967).

Ethylene oxide (oxirane), discovered by A. Wurtz in 1857, is a colorless gas at room temperature with the odor of ether and a stinging taste. At low temperatures, it is a colorless, very mobile liquid, more volatile than ether. Its boiling point is 10.7°C. and freezing point -111.7°C. In mixtures with air (from 3 to 80%) it is explosive, and dissolves well in water, alcohol, ether, and other compounds containing an active hydrogen atom. Ethylene oxide reacts with many organic and inorganic compounds and is stable to the action of higher temperatures. The production of ethylene oxide is carried out by two processes: the action of alkalis on ethylene chlorohydrin, and direct oxidation of ethylene with the aid of catalysts. Ethylene oxide is used in industrial organic synthesis to obtain ethers of glycol, polyglycols, and acrylonitrile, and esters of acrylic acid and their polymers, which are used in the production of plexiglas, synthetic fiber ("orlon"), and synthetic rubber. In addition, ethylene oxide is used in the production of textile materials, wetting agents, and plastic solvents, to control pests in agriculture and at home, and also as a disinfectant in medical practice.

Ethylene oxide is a narcotic which also possesses a high toxicity; the latter may be due to the fact that ethylene oxide reacts in the organism to form formaldehyde or ethylene glycol with the subsequent formation of oxalic acid. It is also possible that ethylene oxide reacting with trimethylamine forms a strongly acting acetylcholine or reacts with the amino groups of proteins (N. V. Lazarev, 1963).

According to Lazarev's data (1963), a man exposed for 10 minutes to an atmosphere containing ethylene oxide vapor showed the following symptoms: violent vomiting, dizziness, and sweet taste in the mouth, with a disturbed cardiac activity for several weeks thereafter.

E. M. Bongard and V. F. Shlyapin (1958) studied the state of health of workers employed in the production of ethylene oxide and coming in direct contact with it. Cases of acute poisoning with ethylene oxide of workers employed in its production were associated with work under emergency conditions, when its concentration was highest. The acute intoxication amounted to a predominant impairment of the neuropsychic sphere.

The Soviet and foreign literature give data on the toxic effect of relatively high concentrations of ethylene oxide. Problems connected with low concentrations of ethylene oxide in atmospheric air and their biological effect have not been studied. We determined ethylene oxide in the air by Ye. Sh. Gronsberg's method modified by N. A. Krylova (M. V. Alekseyeva, 1963). The first stage of the studies was the determination of the threshold of olfactory perception.

Table 1

Results of Determination of the Threshold of Olfactory Perception of Ethylene Oxide

Number of Subjects	Minimum Perceptible Concentration, mg/m^3	Maximum Imperceptible Concentration mg/m^3	Total Number of Tests
2	1,5	1,0	75
3	1,9	1,5	103
3	2,7	1,9	99
2	3,5	2,7	71
2	4,3	3,5	53
6	5,4	4,3	170
2	Over 6,6	—	40

It is evident from Table 1 that the threshold concentration for the most sensitive persons was found to be 1.5 mg/m^3, and the maximum imperceptible concentration, 1 mg/m^3.

In the study of the effect of ethylene oxide on the change of the light sensitivity of the eye it was found that among the most sensitive persons, the minimum active odor threshold concentration was found to be 1 mg/m^3, and the subthreshold concentration, 0.5 mg/m^3 (Table 2, Fig. 1).

Table 2

Change in the Light Sensitivity of the Eye During Inhalation of Ethylene Oxide

Subject	Odor Threshold mg/m^3	Pure air, M (%)	2.4 mg/m^3			1.0 mg/m^3			0.5 mg/m^3		
			M (%)	t	Confidence Factor	M (%)	t	Confidence Factor	M (%)	t	Confidence Factor
Z.Kh.	1,5	205	642	5,5	c	312	2,5	a	178	0,95	o
Ye.B.	1,5	327	427	2,9	a	445	3,6	b	389	1,1	o
L. K.	2,7	159	166	0,39	o	133	1,9	o		—	—

Note. Confidence factor: a - 95%; b - 99%; c - 99.9%.

The effect of ethylene oxide on the electrical activity of the human cerebral cortex was studied on an 8-channel Kaiser electroencephalograph with engineer B. N. Balashev's integrator, using A. D. Semenenko's method. The principle of the latter consists in the following: the subject's electroencephalogram is recorded with simultaneous stimulation with rhythmic light of optimum frequency and varying intensity (ranging from 0.1 to 0.6 J), and the electroencephalogram thus obtained is used to follow the character of reinforcement of the intrinsic potentials of the alpha rhythm. After several tests with pure air, without the subject's knowledge, he is made to breathe the studied gas for 3 minutes in a concentration of imperceptible odor, and the effect of this concentration is evaluated from the degree of change of the amplitude of the intrinsic potentials of the rhythm. The effect of ethylene oxide was studied in concentrations of 2.3, 1.4, 0.65, and 0.3 mg/m^3. The odor threshold for this group of subjects was at a level of 1.9-2.7 mg/m^3. The minimum concentration causing changes in the amplitude of intrinsic potentials of the rhythm in the most sensitive persons was found to be 0.65 mg/m^3. The inactive concentration was 0.3 mg/m^3 (Fig. 2), which we propose as the highest single maximum permissible concentration in atmospheric air (Table 3).

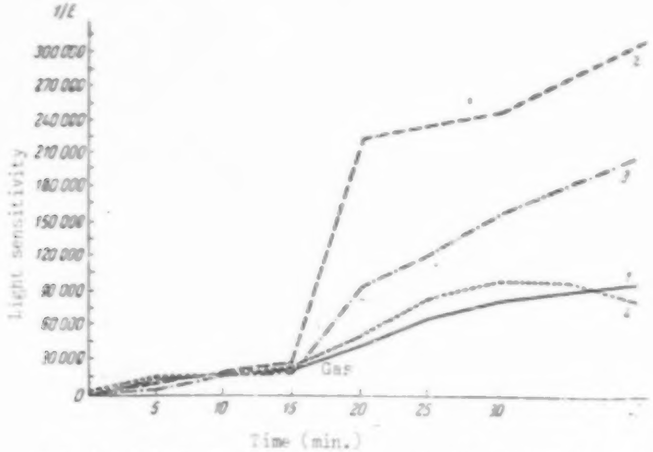

Fig. 1. Effect of ethylene oxide on the light sensitivity of the eye in subject Z. A. Kn.
1 - pure air; 2 - concentration 2.4 mg/m^3; 3 - 1 mg/m^3; 4 - 0.5 mg/m^3.

To validate the mean daily maximum permissible concentration of ethylene oxide, a chronic experiment was carried out on 60 white rats (males) weighing 95-130 g during a continuous 83-day exposure (four groups of 15 animals in each chamber) to the following concentrations of ethylene oxide: group I - 0.3 mg/m^3 (at the level of the recommended highest single concentration); group II - 0.03 mg/m^3; group III - 0.01 mg/m^3, and group IV - control.

The concentration of ethylene oxide was determined daily in each chamber. In group I it amounted to an average of 0.31 ± 0.0069 mg/m^3, in group II, 0.03 ± 0.0016 mg/m^3, and in group III, 0.015 ± 0.0049 mg/m^3. The tests chosen during the chronic exposure were the behavior and weight of the animals, ratio of chronaxy of antagonist muscles, change in the content of chlorides and residual nitrogen in whole blood, change in the content of protein fractions of the blood serum, and anatomico-pathologic and histopathologic examinations at the end of exposure and of the recovery period.

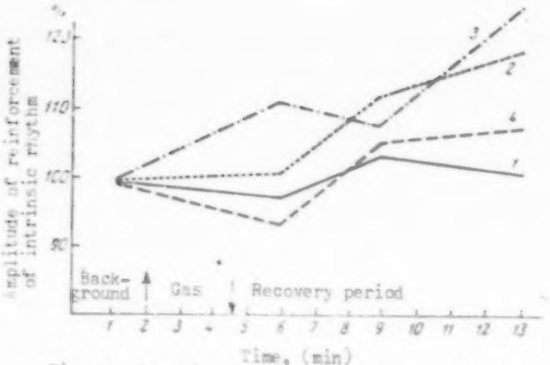

Fig. 2. Dynamics of reinforcement of intrinsic potentiels of the rhythm during inhalation of ethylene oxide in subject R. I.
1 - pure air; 2 - concentration 1.4 mg/m^3; 3 - 0.65 mg/m^3; 4 - 0.3 mg/m^3.

During the entire course of exposure, the animals of all the groups were active, and no statistically significant changes in the weights of the rats were observed in comparison with the control group. The ratio of chronaxy of flexors and extensors of the right hind leg was studied in five rats of each group once every 10 days at the same time and under the same conditions; the response was recorded from the twitch of the animal's foot on an ISE-01 pulse chronaximeter.

Table 3

Combined Results of the Study of the Reflex Effect of Ethylene Oxide on Man.

Response Studied	Concentration of Ethylene Oxide (mg/m^3)	
	Threshold	Sub-Threshold
Olfactory Perception	1,5	1,0
Light Sensitivity of Eyes	1,0	0,5
Electrical Activity of Cerebral Cortex	0,65	0,3

The ratio of chronaxy of antagonist muscles is a sensitive test reflecting the functional shifts in the central nervous system during inhalation of low concentrations of toxic substances (K. A. Bushtuyeva, 1961; V. A. Gofmekler, 1960; Yu. G. Fel'dman, 1962; R. Ubaydullayev, 1961; V. A. Chizhikov, 1963, and others).

In rats of group I (0.3 mg/m^3), a reversed ratio of chronaxies of antagonist muscles appeared in the 8th week of exposure as a result of an increase of the chronaxy of flexors, and remained until the end of exposure. During the recovery period, these changes were normalized. The statistical significance of the changes amounted to 95-99.9%. In animals of groups II and III and the control group, no significant changes in the ratio of the chronaxies of antagonist muscles were noted over the entire course of the tests (Fig. 3).

It is well known that a disturbance of the mineral metabolism takes place in occupational diseases and intoxications, and can be used as a sensitive test, since this shift is proportional to the gravity of the poisoning.

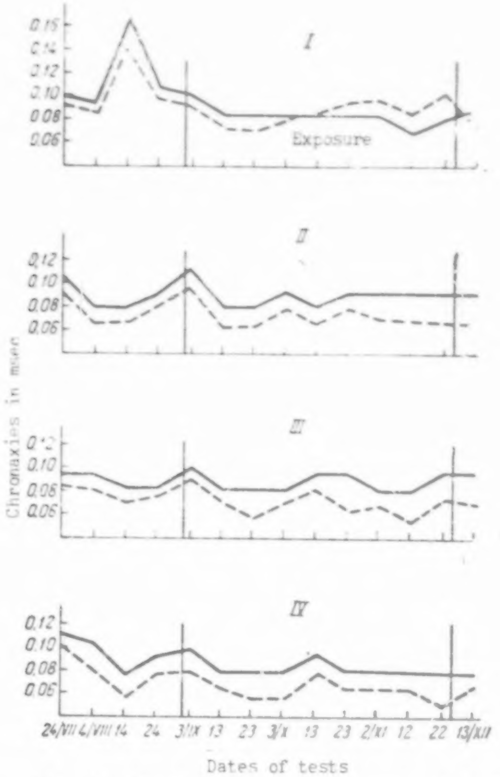

Fig. 3. Change in the ratios of chronaxies of antagonist muscles in the experimental animals under the influence of ethylene oxide.
I, II, III, and IV - test groups; solid line - extensors; dashed line - flexors.

Considering that according to the data of E. V. Zamakov (1946) and N. V. Lazarev (1963) ethylene oxide affects the function of the kidneys and the content of mineral salts in the blood, this being a valuable indicator for determining the harmful influence of many adverse factors, we made an attempt to study the dynamics of chlorides and residual nitrogen in the whole blood of white rats. The chlorides were determined by Levinson's method in five rats of each group, once every 15 days. The method is based on the principle that chlorides reacting with silver nitrate form insoluble silver chloride. The protein-free blood filtrate containing the chlorides is titrated with a solution of silver nitrate in the presence of potassium chromate until there is a color change.

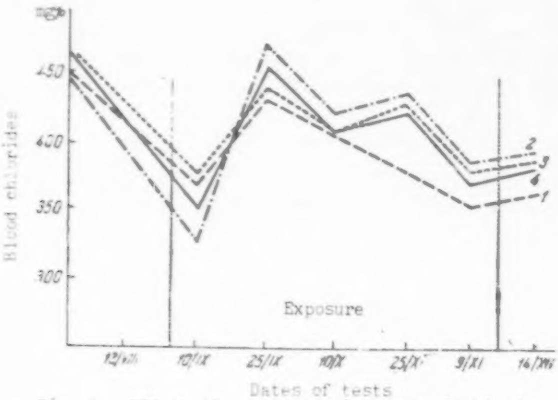

Fig. 4. Effect of ethylene oxide on whole blood chlorides of rats of different groups.
1 - concentration 0.3 mg/m^3; 2 - 0.03 mg/m^3; 3 - 0.01 mg/m^3; 4 - control.

In animals of group I (0.3 mg/m^3), a change in the chloride content of the blood occurred at the end of the 2nd month of exposure and was manifested in a decrease of the chloride concentration to 375.6 mg% as compared with the initial level of 458.3 mg% and 469.5 mg% in the control group. The significance of the indicated changes was 99.9%. During the recovery period, the chloride content returned to normal (Fig. 4).

In rats of group II (0.03 mg/m^3), group III (0.01 mg/m^3) and the control group, no significant changes of the chloride content of the blood were observed. The residual nitrogen in the blood of the animals was determined in Conway cells, in 5 rats of each group after 20 days. The method is based on the principle that an organic compound decomposed by sulfuric acid in the presence of a catalyst forms ammonium sulfate. When alkali is added to the latter, ammonia is liberated. The excess sulfuric acid that does not react with the ammonia is titrated with alkali. From the amount of bound acid one can determine the amount of liberated ammonia and hence that of nitrogen.

In rats of group I (0.3 mg/m^3), a change in the content of residual nitrogen in the blood occurred in the second half of the 2nd month of exposure and was manifested by an increase in residual nitrogen to 56.3 mg % as compared with the initial level (36.4 mg %). The residual nitrogen in the blood of rats of the control group was 38.1 mg %. The significance of the changes was 95%.

During the recovery period, the content of residual nitrogen in the blood was normalized.

In rats of groups II and III, no significant changes in the nitrogen content of the blood were observed (Fig. 5). In the study of the effect of ethylene oxide on the protein fractions of the blood serum in 5 rats of each group with an interval of 20 days, using paper electrophoresis, no significant changes were found in the content of the total protein and protein fractions over the entire course of exposure.

Thus, chronic exposure of white rats to ethylene oxide for 83 days showed that the 0.3 mg/m^3 concentration caused distinct changes in the ratio of chronaxy of antagonist muscles and a change of the chloride concentration and residual nitrogen in the blood.

The 0.03 mg/m^3 ethylene oxide concentration caused no changes in the rats according to all the tests, and we recommended it as the mean daily maximum permissible concentration in atmospheric air.

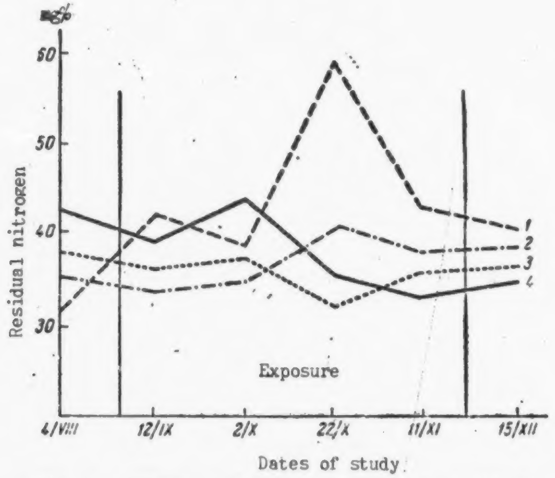

Fig. 5. Effect of ethylene oxide on the residual nitrogen of whole blood of rats of different groups. Notation same as in Fig. 4.

To characterize the pollution of atmospheric air, one of the enterprises where ethylene oxide is produced was surveyed in the summer of 1964. A total of 147 single samples of air were taken at distances from 50 to 500 m from the source of emission on the leeward side (Table 4).

Table 4

Pollution of Atmospheric Air with Ethylene Oxide Around the Plant.

Distance From Source of Emission, m	Number of Samples	Highest Concentration, mg/m³	Distribution of Concentrations in the Samples, mg/m³			
			Above 0.2	0.2–0.15	0.15–0.1	Below 0.1
50	26	0,262	8	17	1	—
100	28	0,230	3	10	11	4
200	31	0,231	5	11	12	3
300	30	0,258	6	16	7	1
500	32	0,1	—	—	1	31

The studies showed that the atmospheric air around this enterprise is polluted with ethylene oxide within a radius of 500 m, although the concentrations found at this distance were substantially below the highest single maximum permissible value which we are recommending.

It should be noted that when the output of the plant is raised or the technology of production is altered (for example, the production of ethylene oxide by direct oxidation), the pollution of atmospheric air with ethylene oxide may increase considerably. However, any conclusions concerning the size of the sanitary-protective zone can be drawn only by also taking into account other components present in the discharges of such enterprises.

Conclusions

1. Ethylene oxide is a poison affecting all the organs and systems, but doing the most damage to the central nervous system.

2. The threshold of olfactory perception of ethylene oxide in the most sensitive persons is 1.5 mg/m^3, the threshold of change of the light sensitivity of the eye is 1 mg/m^3, and the threshold of the effect on the electrical activity of the cerebral cortex is 0.65 mg/m^3.

3. The highest recommended single maximum permissible concentration of ethylene oxide in atmospheric air is 0.3 mg/m^3.

4. Chronic, round-the-clock exposure of white rats to ethylene oxide in a concentration of 0.3 mg/m^3 in the course of 83 days caused changes in the normal ratio of the chronaxy of flexors and extensors and changes in the concentration of chlorides and residual nitrogen in the blood.

5. The mean daily maximum permissible concentration of ethylene oxide in atmospheric air may be specified as 0.03 mg/m^3.

6. The actual pollution of atmospheric air with ethylene oxide a a distance of up to 500 m from the source of emission was determined.

LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1967 bibliography.

SANITARY STANDARDIZATION OF ALPHA-METHYLSTYRENE IN ATMOSPHERIC AIR

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 10, Izdatel'stvo "Meditsina" Moskva, p. 217-225, (1967).

A characteristic feature of the development of modern chemical industry is the growth of the production of synthetic materials, a steady expansion of their assortment, and an increasing quality of the products made from them. An important branch of the chemical industry is the production of synthetic rubber, the volume of which increased 3.4-fold during the last 7 years (1958-1965).

As was shown by our studies, a modern plant producing divinylmethylstyrene synthetic rubber contaminates the atmospheric air with alpha-methylstyrene vapors in a zone of up to 4000 m.

Alpha-methylstyrene is a benzene homolog with one unsaturated bond in the side chain; it is a colorless or slightly yellowish liquid with a boiling point of 162-163°C, and a characteristic odor of naphthalene (N. V. Lazarev, 1963). The toxic effect of alpha-methylstyrene vapors in chronic experiments has been studied by a number of authors.

According to the data of A. I. Korbakova (1961) and E. A. Kapkayev (1963), alpha-methylstyrene vapors in concentrations of 10-40 mg/m^3 and 5-50 mg/m^3 acting for 5-6 hours a day for 6 months affect the functional state of the liver, kidneys, and central nervous system of white rats. Kapkayev also found slight shifts in the peripheral blood of the animals exposed to alpha-methylstyrene vapors in a concentration of 50 mg/m^3. T. A. Blinova (1953, 1954) points out that the inhalation of alpha-methylstyrene vapors in a concentration of 100 mg/m^3 for 6-7 hours in the course of 30 days had a myelotoxic effect on the peripheral blood of rabbits, manifested in erythropenia, leucocytosis with a slight shift to the left, monocytosis, and decrease in the number of reticulocytes. Chronic intoxication with alpha-methylstyrene vapors in combination with divinyl among workers employed in the production of synthetic rubber is manifested by an astheno-vegetative syndrome, cerebral sthenia, and has no distinct specificity. Toward the end of the work week, the workers showed hypotonia and hypothermia (E. A. Kapkayev, 1963; Z. A. Yermolova et al., 1964). To validate the mean daily maximum permissible concentration of alpha-methylstyrene vapors in atmospheric air, we conducted a three-month chronic round-the-clock exposure of white male rats with an initial weight of 140-170 g.

All the rats were divided into four groups of 15 rats each. Group I was exposed to alpha-methylstyrene vapors in a concentration of 5 mg/m^3, corresponding to the maximum permissible concentration for work areas; group II, to 0.5 mg/m^3, and group III, to 0.05 mg/m^3. Group IV was the control. The animals of each group were divided into three subgroups of 5 rats each -- for blood samples and biochemical and physiological examinations. The background indicators were recorded for 1 1/2 months; this was followed by a three-month round-the-clock exposure, and after its completion, by a one-month recovery period. The experimental data obtained were subjected to statistical treatment.

The influence of chronic action of alpha-methylstyrene vapors on the functional state of the central nervous system of the rats was studied by determining the latent time of the unconditioned reflex defensive reaction. We were the first to use this method for the sanitary standardization of atmospheric pollutants, and therefore consider it necessary to dwell in some detail on its procedure in the present report. By latent time of the unconditioned reflex defensive reaction is meant the time necessary for the stimulation to be transmitted along the reflex arc.

Soviet and foreign authors (V. V. Zakusov, 1957; S. I. Gorshkov, 1964; P. Tasaki, 1948) established that the latent time of the unconditioned reflex reaction of laboratory animals is a fine and sensitive indicator of functional shifts in the activity of the central nervous system under the influence of various chemical and physical factors, since the link in which the most pronounced changes of its magnitude take place are the central synapses and cell bodies.

In this respect, as indicated by S. I. Gorshkov (1962), the "latent time of the reflex reaction is much longer than that of the chronaxy characterizing the functional state of any given individual tissue at the instant when it reproduces a single stimulation wave."

An accurate measurement of the latent time of a reflex reaction requires several sets of conditions such as: coincidence of the start of measurement of the latent time with the instant of application of the stimulation, interruption of operation of the counter when the response reaction appears; the stimulation must arise instantaneously without a lag, and must not continue beyond the useful time. These requirements are met by the reflexotonometer, proposed by S. I. Gorshkov (1964) (Fig. 1).

We used the following procedure to determine the latent time of the defensive unconditioned reflex reaction. The rat is set down in the chamber so that its legs touch the parallel metal plates, and the chamber lid, with the counter switch mounted on it, rests on the animal's back. After turning on the time base of the instrument, the threshold intensity of the pulse current, which is the conditioned galvanic skin (pain) stimulus, is selected.

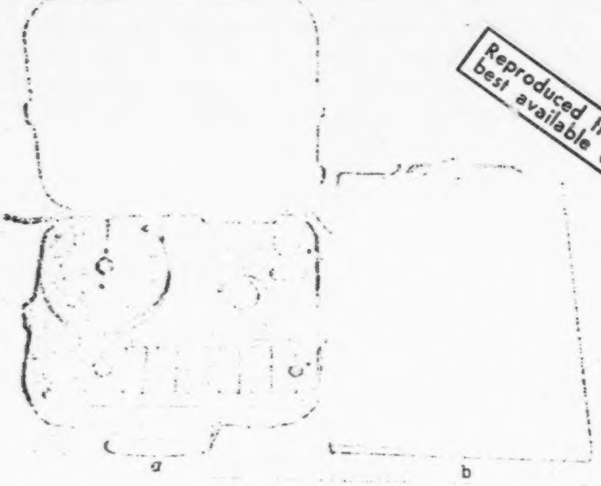

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Fig. 1. Reflexotonometer.
a - counter; b - chamber for animals.

At the threshold current intensity, the rat is startled, causing the switch on the chamber lid to close and the operation of the counter to cease. The time elapsed from the instant the stimulus was applied (location of movable contact) to the instant when the indicator of the counter comes to rest (determined from the scale of the instrument) is the latent time of the defensive unconditioned reflex reaction.

In one series of experiments, five measurements were made at the same current intensity at 2-3 minute intervals (to avoid trace effects). The slight scatter of the data obtained is completely normal and is in itself a functional characteristic of the state of the central nervous system. The arithmetic average of all the measurements is the latent time of the defensive unconditioned reflex reaction.

We studied the influence of alpha-methylstyrene vapors on the change of the latent time of the defensive unconditioned reflex reaction every week on 5 rats of each group. In group I, on the 5th week from the start of exposure, alpha-methylstyrene vapors in a concentration of 5 mg/m^3 caused a statistically significant depression of the functional state of the central nervous system of white rats, manifested in an increase of the latent time of the defensive unconditioned reflex reaction (Fig. 2). These changes were wavy in character and increased until the end of exposure. Toward the end of the recovery period, the latent time of the reaction in the rats returned to normal and did not differ from that of the control group.

Alpha-methylstyrene vapor concentrations of 0.5 mg/m^3 in group II and 0.05 mg/m^3 in group III did not cause any changes in the latent time of the defensive unconditioned reflex reaction. Once every two weeks, we analyzed the blood of the animals and determined the content of nucleic acids, the number of leucocytes and erythrocytes, hemoglobin, and the change in the fluorescent* properties of leucocytes.

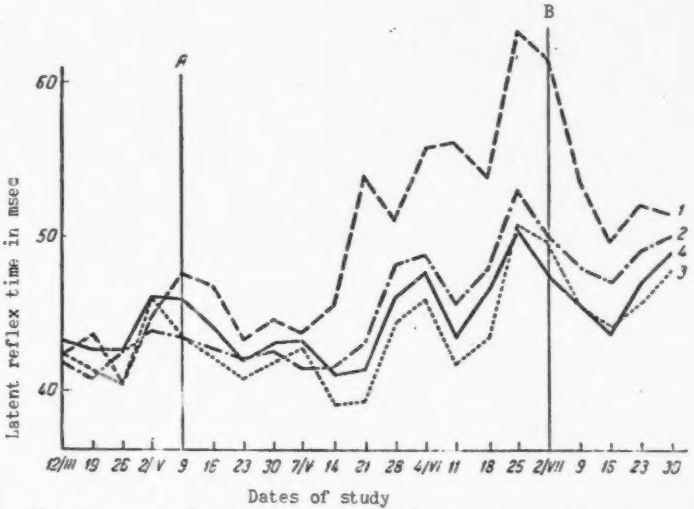

Fig. 2. Change in the latent reflex time under the influence of alpha-methylstyrene vapors.
1 - 5 mg/m^3 (group I); 2 - 0.5 mg/m^3 (group II); 3 - 0.05 mg/m^3 (group III); 4 - control group; AB - period of exposure.

The quantity of erythrocytes and hemoglobin was determined with an erythrohemometer, and the leucocytes were counted in a Goryayev chamber. Alpha-methylstyrene vapors in the concentrations studied did not affect the number of leucocytes and erythrocytes or the content of hemoglobin in the blood of the experimental animals.

The literature contains indications that fluorescent microscopy, without replacing other types of microscopy (ordinary, phase-contrast, electron, interference, ultraviolet, etc.), opens up additional possibilities for biological and medical research (Ye. B. Zakrzhevskiy and L. G. Vasil'yeva, 1963).

The change in the color of the fluorescence of leucocytes under the influence of atmospheric pollutants was studied by A. D. Semenenko (1963).

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* Editor's note: For the Russian use of the terms "luminescent" and "luminescence" in this paper, we have substituted "fluorescent" and "fluorescence", on the basis of the definitions of these terms.

For the sanitary standardization of toxic substances in atmospheric air, fluorescent microscopy was first used by M. I. Gusev and K. N. Chekalinov (1963) in a study of pentene in a chronic experiment on animals.

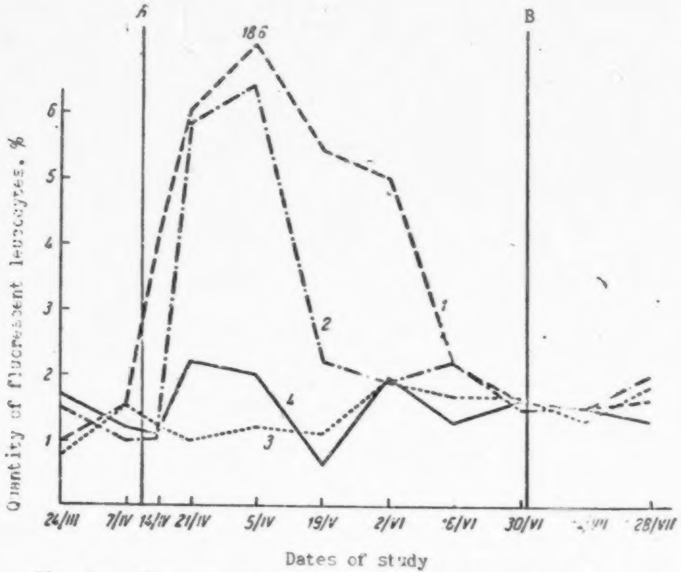

Fig. 3. Effect of alpha-methylstyrene on the content of fluorescent leucocytes with altered fluorescence in the blood.
Notation same as in Fig. 2.

We studied the changes in the color of fluorescent leucocytes in vitally fluorochromed liquid preparations (the fluorochrome used was a 1:100,000 solution of acridine orange in saline). The source of exciting light was an ON-18 fluorescent illuminator.

Alpha-methylstyrene vapors in concentrations of 5 and 0.5 mg/m^3 caused a change in the color of the fluorescence of leucocytes from green (normal) to yellow and yellowish-orange. In rats of group I, these changes were more pronounced and continued over a longer period of time. In rats of group III (0.05 mg/m^3), the number of fluorescent leucocytes with altered fluorescence did not differ from that of the control (Fig. 3).

At the present time, the attention of many researchers is focused on nucleic acids, whose biological function is related to the processes of protein biosynthesis, mechanisms of normal and pathological growth and development, heredity, and biological specificity.

Nucleic acids are high-molecular-weight organic compounds present in all biological systems and cells of the organism and are an integral part of them.

However, the state of nucleic acids in the organism under the influence of toxic substances in atmospheric air has not been studied in connection with sanitary standardization.

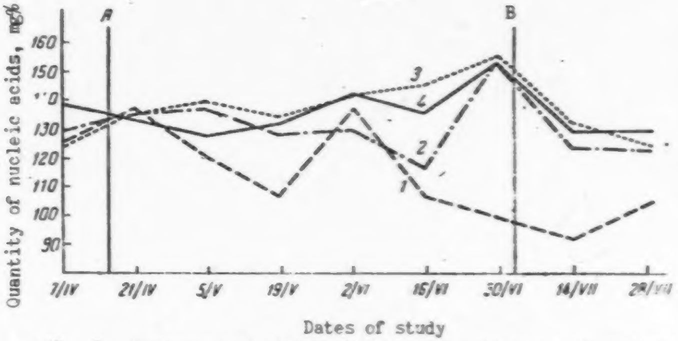

Fig. 4. Influence of alpha-methylstyrene vapors on the content of nucleic acids in the blood.
Notation same as in Fig. 2.

In our investigations, we used a spectrophotometric method of quantitative determination of total nucleic acids (RNA and DNA) proposed by A. S. Spirin (1958). The method is based on the extraction of nucleic acids with hot perchloric acid followed by photometry in the ultraviolet at two wavelengths (270 and 290 mμ). In the quantitative determination of nucleic acids in the blood of the animals, we used the procedure proposed by P. V. Simonov (1960, 1962).

The results of our studies (Fig. 4) show that alpha-methylstyrene vapors in a concentration of 5 mg/m^3 (group I) caused a decrease in the content of total nucleic acids as early as the 4th week of exposure. Then, after a short remission period, a second decrease took place which remained abnormal until the end of the recovery period. In rats of groups II and III, the quantity of nucleic acids in the blood did not differ from that of the control.

We evaluated the influence of the chronic action of alpha-methylstyrene vapors on the porphyrin metabolism from the change in the content of coproporphyrin in the daily diuresis of 5 rats once every two weeks. The amount of coproporphyrin was determined by a procedure described by M. I. Gusev and Yu. K. Smirnov (1960).

Alpha-methylstyrene vapors in a concentration of 5 mg/m^3 (group I) caused a decrease of coproporphyrin in the urine as early as the second week of exposure. This decrease lasted during the entire period of exposure and remained abnormal until the end of the recovery period. In animals of group II, the changes were less pronounced. In rats of group III, there were no changes in porphyrin metabolism in comparison with the control (Fig. 5).

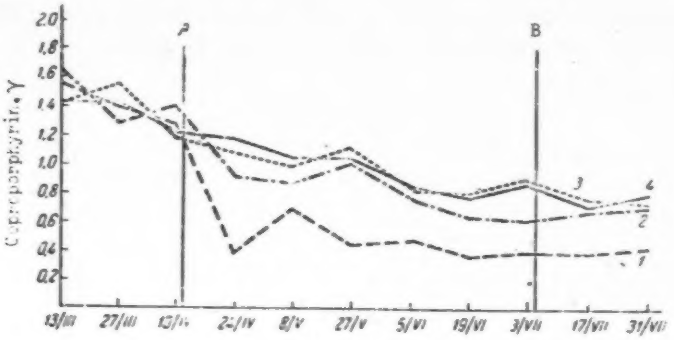

Fig. 5. Influence of alpha-methylstyrene vapors on the content of coproporphyrin in the urine.
Notation same as in Fig. 2.

Alpha-methylstyrene vapors in concentrations of 5, 0.5, and 0.05 mg/m^3 had no influence on the behavior and weight of the animals during the chronic experiment.

After the completion of exposure, 5 rats of each group were killed. The results of anatomico-pathologic and histologic examinations of the organs showed that the alpha-methylstyrene vapors in the concentration of 5 mg/m^3 caused changes in the lungs, kidneys, heart, and liver.

Thus, the three-month chronic round-the-clock exposure of white rats leads to the following conclusions:

1. Alpha-methylstyrene vapors in a concentration of 5 mg/m^3 and below had no effect on the behavior and weight of the animals or on the total blood picture (quantity of leucocytes, erythrocytes, hemoglobin).

2. A 5 mg/m^3 concentration of alpha-methylstyrene vapors caused a depression of the central nervous system (increase of the reflex time), a change in the biochemical reactions (decrease in the content of nucleic acids in the blood and coproporphyrin in the urine), and a change in the fluorescent properties of the leucocytes.

The functional changes were confirmed by histopathologic changes in the lungs, heart, liver, and kidneys.

3. Alpha-methylstyrene in a 0.5 mg/m^3 concentration caused only a change in the fluorescent properties of the leucocytes; this concentration was found to be inactive in all the tests.

4. The mean daily maximum permissible concentration of alpha-methylstyrene vapors for atmospheric air may be established at the level of the accepted highest single concentration, 0.04 mg/m^3, which was found to be inactive in chronic exposure of the experimental animals.

LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1967 bibliography.

SANITARY VALIDATION OF THE MAXIMUM PERMISSIBLE CONCENTRATION OF DIVINYL IN ATMOSPHERIC AIR

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From Akademiya Meditsinskikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 10, Izdatel'stvo "Meditsina" Moskva, p. 33-54, (1967).

One of the principal monomers in the production of synthetic rubber is divinyl (1, 3-butadiene). A diene hydrocarbon with two conjugated double bonds, it polymerizes readily, and is therefore widely used in the production of different varieties of synthetic rubber.

Under ordinary conditions, divinyl is a gas with a pungent, unpleasant odor; its specific gravity relative to air is 1.87; at a temperature of -4.5°, divinyl condenses into a transparent, colorless, volatile liquid.

Divinyl is sparingly soluble in water and highly soluble in acetone, benzene, and amyl acetate (A. A. Petrov, 1953), and less soluble in ethanol (P. Vatsulik, 1960).

In high concentrations, divinyl is regarded by many authors (V. A. Pokrovskiy, 1955; V. S. Freydlin, 1935; L. F. Larionov, 1954; E. N. Levina, 1948; Henderson and Haggard, 1930, and others) as a narcotic with a marked irritant effect on the muccous membranes and blood-forming organs.

The maximum permissible concentration for industrial buildings is 100 mg/m^3.

Among workers affected by divinyl in the course of production one sees a number of functional disturbances of the central nervous system (rapid fatigability, headache, drowsiness), disturbances of the upper respiratory tract (rhinitis, laryngitis, pharyngitis), conjunctivitis, changes in the composition of peripheral blood, dyspeptic phenomena, decrease of arterial pressure, etc. (S. B. Glauberman, 1957; L. I. Gefter and Ye. D. Shul'man, 1935; S. D. Polyak, N. I. Pil'man, A. I. Zaretskaya, and S. M. Kleyn, 1935; A. A. Yegunov, 1940; E. N. Levina, 1948; A. A. Orlova and G. N. Mazunina, 1960; L. F. Larionov, 1954).

Most of the symptoms disappear after contact with divinyl is discontinued.

Animals subjected to the chronic action of divinyl were found to have slight pathologic changes: enlargement of mucous cells in the bronchial epithelium, small-cell infiltration around them, foci of emphysema in the lungs, hyperplasia of the bone marrow with enlargement of young forms; in the spleen, enlargement of myeloid elements in the red pulp; and in the lymphatic nodes, indications of irritation (L. F. Larionov, 1954).

The majority of the enumerated studies describe the effect of divinyl on the human organism when divinyl is present in substantial concentrations in the air of plant buildings. In the literature available to us, we found no data on the effect on the organism of low concentrations of divinyl present in atmospheric air.

However, pollution of atmospheric air with divinyl takes place as a result of production of synthetic rubber. The widespread use of divinyl in the production of synthetic rubber and the possibility of pollution of the air with it make it necessary to study the effect of its microconcentrations and to arrive at a sanitary validation of its maximum permissible concentration in atmospheric air. This constitutes the subject of the present paper.

In order to determine low divinyl concentrations in air under experimental conditions and in atmospheric air, Candidate of Biological Sciences M. D. Manit developed a spectrophotometric method of determining microquantities of divinyl and determined the optimum parameters for the collection of air samples. This method makes it possible to determine divinyl in atmospheric air under natural conditions in the presence of other components of production of synthetic rubber: α-methylstyrine, isopropylbenzene, butane, and butylenes.

The threshold of olfactory perception was determined by a common procedure (V. A. Ryazanov, K. A. Bushtuyeva, and Yu. A. Novikov, 1957), but because divinyl is a very volatile gas under ordinary conditions, some modifications affecting the dispensing of the gas in the system were introduced into this procedure.

The experimental mixture of the gas and air in the required concentration was obtained in steel (oxygen or nitrogen) cylinders of 40 l capacity by evacuation followed by filling with a small amount of gaseous divinyl. After divinyl was introduced into the steel cylinder, it was diluted and uniformly mixed with a large volume of compressed air pumped up to a pressure of 20-30 atm or higher. A constant divinyl concentration was obtained by allowing the mixture to leave the cylinder through a reducing valve. The divinyl concentration in the steel cylinder was determined by calculation and with an SF-4 spectrophotometer in the gas phase. The divinyl concentration in the inhalation cylinders which was required for the studies was obtained by adjusting the feed rate of the prepared mixture leaving the steel cylinder and by diluting it with pure air forced into the inhalation cylinders by air compressors at a rate of 15-20 l/min. This method of supplying the gas mixture ensured a uniformity and constancy of the divinyl concentrations in the inhalation cylinders. By using a reducing valve to adjust the feed rate of the divinyl-air mixture leaving the steel cylinder, one can obtain the desired gas concentration in the system. The feed rate of the gas mixture is measured with a float-type flowmeter mounted past the reducing valve.

The study of the threshold of olfactory perception involved 16 persons aged 18-36 years without any irregularities in the condition of the ear, nose, and throat. All the subjects were first familiarized with the odor of divinyl. The pungent and unpleasant odor of the gas is readily distinguishable and is well remembered.

The concentration of divinyl in the inhalation cylinders was checked twice (before and after each test).

A total of 336 tests were conducted at divinyl concentrations of 6.0, 5.2, 4.8, 4.4, 4.2, 4, and 3.8 mg/m^3 (Table 1).

Table 1

Number of Subjects	Minimum Perceptible Divinyl Concentrations mg/m^3	Maximum Imceptible Divinyl Concentrations mg/m^3	Total Observations
6	4,0	3,8	150
6	4,2	4,0	126
3	4,4	4,2	48
1	4,8	4,4	12

From the data of Table 1 it follows that the threshold of olfactory perception of divinyl established in the experiment is 4 mg/m^3.

Studies of the effect of divinyl on the light sensitivity of the eye were conducted in accordance with the procedure adopted by the Committee on Sanitary Protection of Atmospheric Air. They included four subjects aged 20-35 years with a normal vision and sense of smell.

Tests of the light sensitivity were carried out after a 10-minute dark adaptation. In the first 4-6 days, the light sensitivity of the eye was determined during inhalation of pure air from the cylinder. In the following days, between the 15th and 20th minute of darkness, divinyl in subthreshold odor concentrations was supplied to the inhalation cylinder.

The effect of the gas in the corresponding concentrations was checked daily for three days on all the subjects.

The odor of the first concentration checked, 4.2 mg/m^3, was imperceptible to the subjects. Like the 4 mg/m^3 concentration, it caused in all the subjects a substantial increase of the light sensitivity on the 20th minute as compared to pure air. These differences were statistically significant in all the subjects.

The 3.8 mg/m^3 concentration caused an increase of the light sensitivity in two subjects (P. A. K. and U. A. F.), but this increase was statistically significant in only one subject. In the others, even when the curve of the light sensitivity of the eyes was slightly changed, the results obtained were statistically insignificant.

At the 3.6 mg/m^3 concentration, the gas did not cause any changes in the light sensitivity (Fig. 1).

Thus, the threshold of the reflex effect of divinyl on the light sensitivity of the eye is 3.8 mg/m^3 and is close to the threshold of olfactory perception.

The method of development of the electrocortical conditioned reflex (K. A. Bushtuyeva, Ye. F. Polezhayev, and A. D. Semenenko, 1960) was used to determine the threshold of the reflex effect of divinyl on the central nervous system.

The tests were conducted on a Kaiser electroencephalograph.* At the start of the experiment, 24 persons were studied, of which 4 (2 men and 2 women) aged 18-30 years with a well-defined alpha rhythm and its distinct desynchronization to light were chosen for the observations.

All the subjects participated for the first time, in such an experiment, and during their training gave well-defined, distinct encephalogram tracings. The tests were conducted in the middle of the day for each person at the same time every day.

Divinyl concentrations of 3.8, 3.6 and 3 mg/m^3 were checked. At 3.8 mg/m^3, a conditioned electrocortical reflex was formed in two subjects: in A. S. on the first day on the 6th pairing and on the second day on the 5th pairing. The reflex was preserved until the 12-16th pairing. This concentration of divinyl had a similar effect on the third and fourth day of the study.

Gas in concentrations of 3.6 and 3 mg/m^3 in a given subject did not bring about the formation of the conditioned electrocortical reflex.

In G. Kh., a conditioned electrocortical reflex was formed on the 5th-17th pairing at the 3.8 mg/m^3 divinyl concentration and very indistinctly at 3.6 mg/m^3; no effect of divinyl was detected at the 3 mg/m^3 concentration.

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* The study was conducted in the department of communal hygiene of the Central Institute for Advanced Training of Physicians under the direction of Doctor of Medical Sciences K. A. Bushtuyeva.

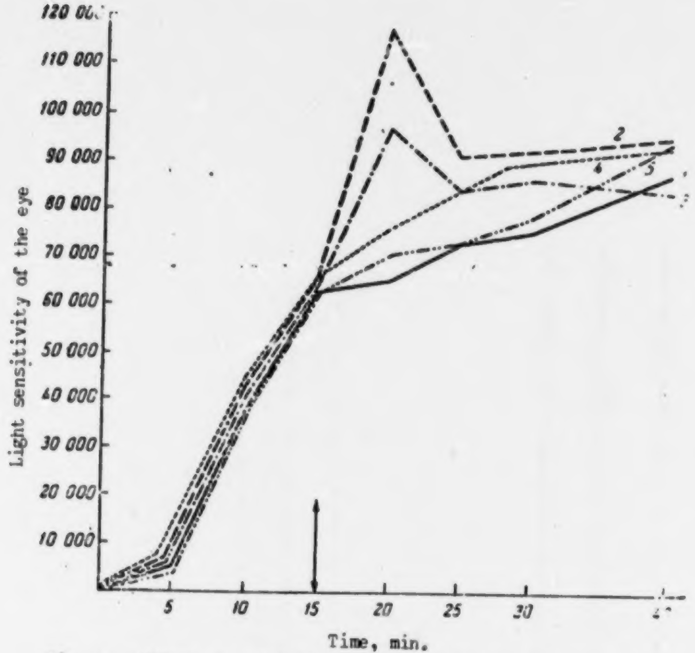

Fig. 1. Effect of inhalation of divinyl in various concentrations on the light sensitivity of the eye in subject P. A. K.
1 - pure air; 2 - concentration 4.2 mg/m^3; 3 - 4 mg/m^3; 4 - 3.8 mg/m^3
5 - 3.6 mg/m^3.

Table 2

Light Sensitivity of the Eyes During Inhalation of Divinyl (on the 20th minute in percent of 15th minute)

Subject	Pure Air	Divinyl Concentration, mg/m^3							
		4.2		4.0		3.8		3.6	
		%	Confidence Factor,%	%	Confidence Factor,%	%	Confidence Factor,%	%	Confidence Factor,%
U.A.F.	128	156	99,9	131	99,0	130	75,0	128	No Differences
S.G.V.	111	129	99,9	116	90,0	110	50,0		
P.A.K.	112	176	99,9	146	99,9	120	98,0	112	No Differences
D.Ye.N.	123	222	99,9	128	50,0	121	50,0		

In R. P. and G. G., a conditioned electrocortical reflex was developed at 3.6 mg/m^3 (Fig. 2); the 3 mg/m^3 concentration was found to be inactive (Fig. 3).

Thus, the 3 mg/m^3 divinyl concentration was found to be inactive in all the subjects.

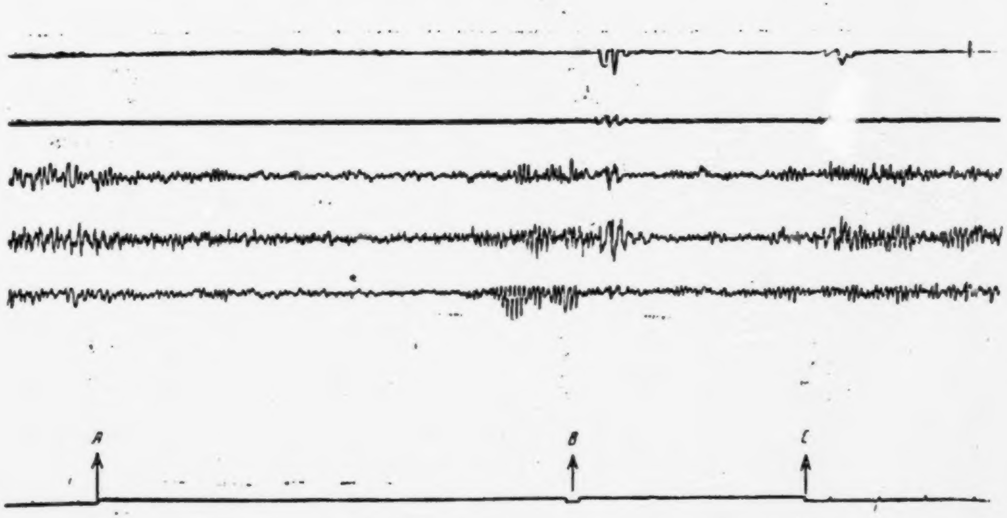

Fig. 2. Electroencephalogram of subject R. P. Conditioned reflex desynchronization of alpha rhythm on the 8th pairing during inhalation of divinyl in a concentration of 3.6 mg/m^3;
AC - inhalation of gas concentrations studied; BC - with light turned on.

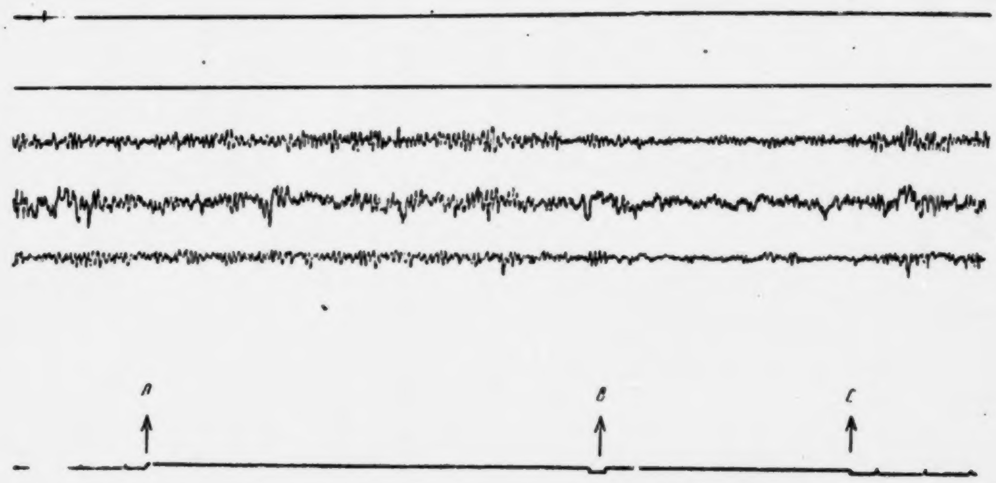

Fig. 3. Electroencephalogram of subject R. P. Absence of conditioned reflex desynchronization of alpha rhythm during inhalation of divinyl in a concentration of 3 mg/m^3.
Notation same as in Fig. 2.

Comparing the data on the threshold of action of divinyl on olfactory perception (4 mg/m^3), on the light sensitivity of the eye (3.8 mg/m^3), and on the electrical activity of the brain (3.6 mg/m^3), we can propose 3 mg/m^3 as the highest single maximum permissible concentration of divinyl in atmospheric air.

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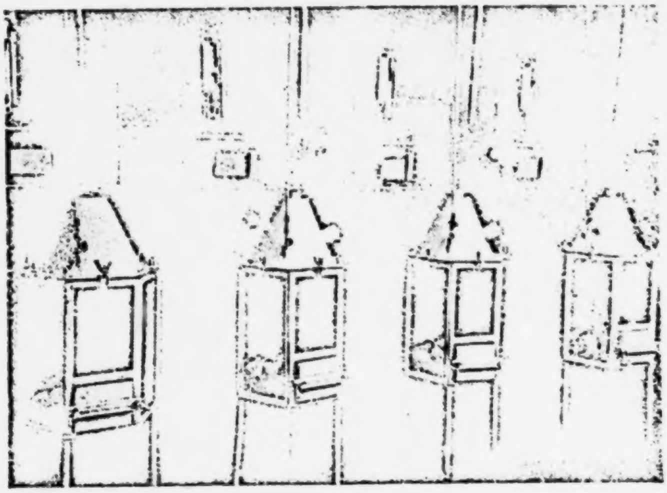

Fig. 4. General view of exposure chambers with dispensing devices.

In order to explain the resorptive action of divinyl in continuous, long-term inhalation and to validate its mean daily maximum permissible concentration, white rats were subjected to a round-the-clock exposure to divinyl for 81 days (from 19 May to 7 August 1964).

The exposure was carried out in four chambers with a volume of 0.1 m^3 each (Fig. 4); the air was forced into the chambers by removable VPP-4 air compressors at the rate of 30 l/min. Divinyl in the gaseous state was added from steel cylinders to pure air.

Group I was the control (pure air). A concentration of 1.03 ± 0.006 mg/m^3 was maintained in group II, 3.08 ± 0.006 mg/m^3 in group III, and 30.8 ± 0.075 mg/m^3 in group IV.

The concentration of divinyl in the chambers was checked daily with an SF-4 spectrophotometer in a gas cell and by sampling on a solid sorbent.

During the exposure, the temperature and humidity in the chambers were also recorded. These indices were comparatively stable.

The animals were fed in the chambers twice a day, the food assortment being varied (boiled meat, milk, groats, bread, oats, grass, vegetables, and cod-liver oil once a week).

To evaluate the condition of the animals and possible shifts in the course of prolonged exposure to divinyl, various tests were chosen. Their list and schedule are given in the text.

Before the start of the exposure, the animals were in quarantine, then under observation for two weeks, and subjected to background studies according to the chosen tests to determine the subsequent shifts.

Each chamber (group) contained 14-15 rats (males) of the same average weight in each group.

At the end of the exposure, some of the animals were killed immediately by decapitation, and some remained under observation for two weeks. At the end of the recovery period, the remaining animals were also killed and their organs were subjected to histopathological examination.

In the course of exposure of the rats, the following changes in the condition of the animals and functional shifts were observed.

General condition and weight of the animals. In the first days of exposure, animals of group IV showed a certain agitation that was obviously due to the irritant action of the gas. These symptoms were less pronounced in group III.

Rats of the control group and groups II and III during the entire course of exposure were healthy, active, ate well, and their condition did not show any difference whatsoever. They gained weight normally. However, in group IV at the start of the third month of exposure, there was a distinct decrease in the activity of the animals, a reduction of the appetite, and a weight lag relative to the animals of the control and other groups (Fig. 5). The weight differences reached statistically significant values only toward the end of exposure. After the exposure, animals of group IV began to gain weight rapidly.

State of peripheral blood. Studies of the blood were made on 5 rats of each group once every 14-15 days at a given time of day. The blood to be analyzed was taken from a caudal vein, and the number of erythrocytes, hemoglobin (on a photoelectric erythrohemometer), and reticulocytes* were determined. Changes in the red blood of rats in the course of exposure were not well-defined.

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* The reticulocyte smears were prepared according to Zhgun's procedure (1962).

Simultaneously with the above tests on red blood, the number of leucocytes was counted according to a common procedure, and the leucocyte count was determined.

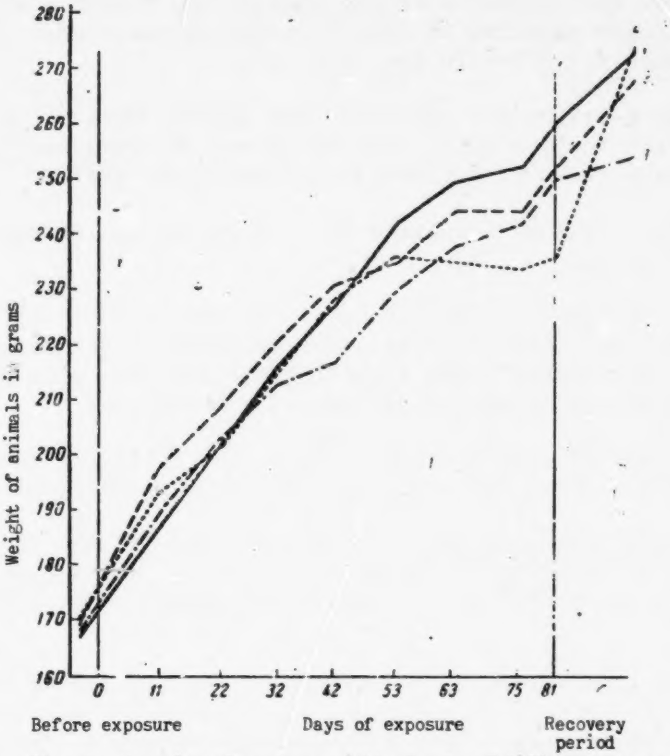

Fig. 5. Dynamics of average weight of rats of different groups in the course of round-the-clock exposure to divinyl.
1 - control group; 2 - group II (concentration of divinyl 1 mg/m^3); 3 - group III (3 mg/m^3); 4 - group IV (30 mg/m^3).

At the end of exposure, a certain degree of leucocytosis was observed only in rats of group IV, but a statistically significant difference was noted only on the 80th day of exposure. Against a background of moderate leucocytosis were visible some shifts in the percent content of lymphocytes and stab-nucleated, [trans. note: leucocytes] but these differences were statistically insignificant.

Activity of whole blood cholinesterase. The cholinesterase activity is related to the function of the mediators and unquestionably affects the state of the central nervous system and the transfer of a neuromuscular impulse.

The activity of cholinesterase determines the rate of hydrolysis of acetylcholine, and its inactivation promotes the accumulation of acetylcholine.

In the majority of studies on the effect of noxious substances on the animal organism, appreciable shifts in cholinesterase activity were observed.

In our studies, the determination was made in five rats of each group twice before the exposure and then once every 14-15 days in the course of exposure according to the method of Limperos and Ranta (1953) modified by Nicholls (1955). The method was described by Merewether (1960). The index of cholinesterase activity was the time of acetylcholine hydrolysis, which was determined visually.

The changes in cholinesterase activity are clearly evident only in group IV (Fig. 6). Statistically significant differences were noted in this group on the 7th, 38th, 56th, 73rd, and 81st day of exposure. After the exposure was completed, the indices of cholinesterase activity became similar to the initial ones.

Optical properties of the blood serum. The ability of different substances to absorb light selectively in a given region and at a particular wavelength also characterizes the blood serum. Kamenetskaya (1960) cites data on the ultraviolet absorption by the blood serum of rabbits at wavelengths from 230 to 300 mμ with a maximum absorption at 275 mμ. This selective absorption is explained by the presence of the aromatic amino acids tyrosine (with λ_{max} 280 mμ) and tryptophan (with λ_{max} 275 mμ) in the blood serum proteins. Kamenetskaya concludes that changes in the absorption spectra of the blood serum are due to an alteration of the serum proteins, and in particular, of its tyrosine and tryptophan content.

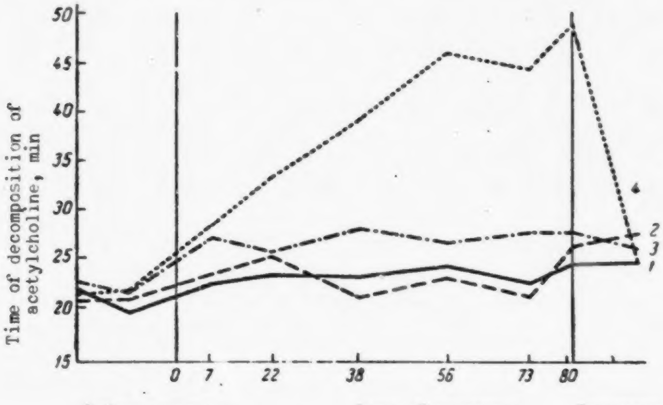

Fig. 6. Effect of divinyl on the activity of whole blood cholinesterase of rats in different groups during round-the-clock exposure to divinyl. Notation same as in Fig. 5.

Similar data are cited by A. D. Semenenko (1963), who studied the effect of low concentrations of aniline on the optical properties of blood serum in rats. She also noted that the absorption maximum and minimum of the serum were located at 278 and 250 mμ respectively.

The ratio of the optical density at λ_{max} to the optical density at λ_{min} in healthy rats ranges from 1.8 to 1.93 (absorption index).

Before starting the exposure of the rats to divinyl, the spectral characteristic of the blood serum of 20 rats (5 in each group) was recorded on an SF-4 spectrophotometer in quartz cells with a light path of 10 mm. The serum was diluted 500 times with saline solution.

The absorption maximum and minimum were located at 278 and 250 mμ. However, in the near ultraviolet (around 205-208 mμ) there was also a considerable absorption. We did not use this peak, since work in the near ultraviolet region (at 205 mμ) is beyond the sensitivity range of the SF-4.

The absorption index of the serum before the exposure was 1.7-1.85. As early as the 9th day of exposure it rose to 2.07-2.36 in 3 rats of group IV, and on the 30th day it amounted to 2.03-2.36 in all the rats of this group, remaining in this range until the end of exposure and slightly lower during the recovery period. No noticeable shifts in the absorption index were found in rats of the remaining groups.

Despite the change in the absorption index of the blood serum in rats of group IV, the position of the absorption bands remained unchanged. Apparently, the divinyl caused shifts in the content of aromatic amino acids, which changed the absorption of light by the serum mainly by increasing the optical densities at λ_{max} 278 mμ. The general intoxication of the organism because of the inhalation of divinyl in rats of group IV should have led to an increase in the content of a series of amino acids in the blood.*

The mechanism of the shifts taking place should unquestionably be subjected to a closer analysis, but the high sensitivity of the spectrophotometric method of studying the optical properties of the blood serum makes it possible to detect the most delicate shifts in its protein composition.

During the two weeks of the recovery period, the index remained high without reaching the original values. In rats of groups II and III in the course of the entire exposure, no substantial shifts were found either in the absorption band or in the optical densities and absorption index.

Thus, those changes in optical properties of the blood serum which were observed in rats of group IV in the course of their round-the-clock exposure to divinyl attest to shifts in the state of serum proteins due to the action of divinyl in a concentration of 30 mg/m^3. At the same time, the absence of shifts in animals of the control group and groups II and III shows the divinyl concentrations of 1 and 3 mg/m^3 to be inactive according to this test.

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* Simultaneously with the detection of the influence of divinyl on the optical properties of the blood, in collaboration with T. I. Lyutikova, we carried out a similar study involving exposure of white rats to cracking gas, and also obtained distinct shifts in the absorption index.

Comparing the data obtained from the investigation of the blood in the course of exposure of rats in group IV, one can observe appreciable shifts in the leucocyte count, in the decrease of cholinesterase activity, and in the change of the optical properties of the blood, along with an absence of marked changes in the red blood cells.

Arterial pressure. The feasibility of evaluating the effect of divinyl on the animal organism in the course of a prolonged round-the-clock exposure is related to the earlier-described hypotensive effect of divinyl under industrial conditions. A hypotensive effect is also displayed by the second monomer in the production of synthetic rubber, α-methylstyrene (E. A. Kapkayev, 1963).

The arterial pressure of rats was determined by the bloodless method (V. M. Chernov, 1947; A. Kh. Kogan, 1959) in a tail plethysmograph with a glass cuff applied at the base of the tail; the pressure was recorded on a sphygmomanometer, and the oscillations, on a water manometer.

The pressure was measured in 5 rats of each group. First, before the start of exposure, in the course of training measurements, the animals became accustomed to the experimental situation, then the initial level of arterial pressure was determined.

Subsequent changes were carried out in the course of exposure once every 3 weeks during evening hours.

The level of arterial pressure in the rats was very stable in all the groups before the start of exposure and during the latter over the course of more than two months (Fig. 7). However, on the 72nd day of exposure, the average level of arterial pressure in rats of group IV decreased from 90-93 mm Hg to 75-77 mm Hg and remained at this level until the end of exposure and also during the recovery period. This decrease was statistically significant beginning with the 72nd day.

The arterial pressure in rats of the control group and groups II and III did not change. Thus, according to this test, the active divinyl concentration is 30 mg/m^3.

Total motor activity of the animals. In order to evaluate the effect of divinyl on the central nervous system and determine whether stimulation or inhibition processes predominate in the cerebral cortex, we chose the method of objective recording of the total motor activity of the animals, based on the principle of actography.

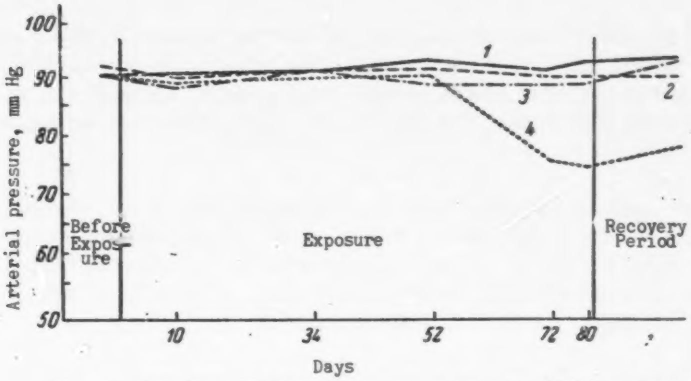

Fig. 7. Dynamics of the blood pressure of rats of different groups in the course of round-the-clock exposure to divinyl. Notation same as in Fig. 5.

The method of actography has been used to record the motor activity and conditioned reflex activity in studying the daily periodicity in the animal organism (A. D. Slonim, 1953; N. I. Kalabukhov, 1940; Ya. B. Maksimovich, 1954; O. S. Kushakovskiy, 1954).

The impulses were recorded by means of a kymograph connected to air-filled capsules, cushions, electromechanical counters, etc.

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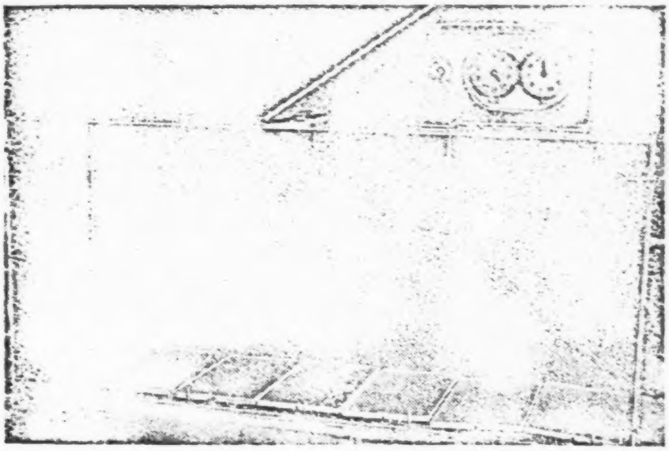

Fig. 8. Chamber for studying the total motor activity of the animals.

Special plexiglas chambers with a sectional bottom made up of seven movable plates mounted at the center on semiaxes were prepared for this study (Fig. 8). When the pressure on one of the halves of the plate exceeds 0.5 g/cm^2, an electric contact located under the plate is closed, making it possible to record each movement of the animal in the chamber. The contracts were connected to an electromagnetic mechanical counter plugged into a DC line with a voltage of 12 V. During the study, the counter records all the incoming pulses.

The experiment involved the use of three such chambers with counters; a rat was placed in each chamber for three hours. Each rat was studied no fewer than three times a week. In each group, the actography was carried out on 5 rats. First, before the exposure, the rats were trained for a few days to get accustomed to the situation in the chambers. On the 2nd-3rd day, the rats adapted to the situation, and their behavior in the chambers became stabilized. A response to the changed situation was manifested only during the first 5-10 minutes after they were placed in the chambers. To eliminate this effect, the counters were turned on 15 minutes after the animal was placed in the chamber. For 3-4 days, the background of the total motor activity was determined; the average figures for the total motor activity of each rat during the initial period were taken as 100% and comprised the initial background of the study. Subsequently, data on the motor activity of the animals for the weeks of exposure in each group were summed up. The final data are reflected in Fig. 9. The decrease in the motor activity of animals in group IV as compared with the control, a decrease which began in the 7th week of exposure, slightly preceded the changes in weight, general condition, and arterial pressure. The method of objective recording made it possible to expose these shifts before the above-mentioned remaining changes were manifested. The decrease of the total motor activity in rats of group IV lasted until the end of the exposure and during the recovery period.

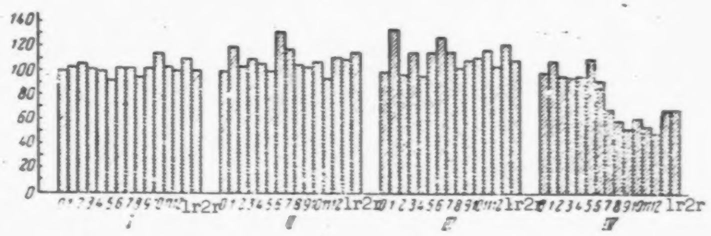

Fig. 9. Change in the motor activity of rats in the course of round-the-clock exposure to divinyl (total based on weeks in percent of original activity).
I-IV - animal groups; 0 - background; 1-12 - weeks of exposure; 1r-2r - weeks of recovery period.

The differences obtained in group IV as compared with the control were statistically significant. In groups II and III, no substantial differences in motor activity of the animals as compared with the control were detected. According to this test, the active divinyl concentration was found to be only 30 mg/m^3.

Data of histopathological study.* The specimens used in the study were sections taken from the liver, kidneys, heart, spleen, brain, and femur bone marrow. In addition, the nasal mucosa in the anterior compartment and at the nasopharynx level were studied.

The sections of organs were embedded in paraffin (from bone tissue after preliminary decalcification). The paraffin sections were stained with hematoxylin-eosin. In addition, sections of the brain were embedded in celloidin and the sections prepared from them were stained by the Nissl method.

The studies performed led to the following findings: the nasal mucosa was not completely intact in animals of the control group either. In the latter, focal and in some cases diffuse infiltrates were observed along with the preservation of epithelium.

These changes may be caused by the reaction of the animals to external factors (mechanical stimuli because of the presence of a large number of animals in the chamber, air humidity, etc.).

In animals of group III (3 mg/m^3), a loosening of the mucosal epithelium was manifested more frequently than in the control group. An even greater difference from animals of the control group was observed in animals of group IV (30 mg/m^3). Most of these animals showed a marked inflammatory infiltration of the mucosa; in three animals, this infiltration was diffuse with massive exudation in the nasal cavity.

In studies of the lungs of rats in group II (1 mg/m^3), no difference was found as compared with those of the control group. In the majority of rats of group III, against a background of a well-developed lymphoid tissue of the lungs, there was a moderately well-defined interstitial pneumonia and small foci of atelectasis and emphysema.

Almost all the rats of group IV had small foci of atelectasis and emphysema, and also a well-developed hyperplastic lymphoid tissue. Many animals showed the presence of interstitial infiltration and perivascular edema, and also interstitial pneumonia.

The brain did not show any difference between the control group and the remaining animals of all three groups. The structure of the bone marrow was the same in all the groups. Accumulations of hemosiderin were observed in the red pulp of the spleen of animals in group IV only.

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* The slide mounts were studied and described by Docent V. V. Veselova.

In some animals of group IV, a thinning of the hepatic lobes with an almost total absence of clear cells, which predominate in the liver of the rats of the first three groups, was noted. It must be postulated that these are cells rich in glycogen.

In some rats of group IV, swelling of the endothelium of the sinusoids and disorganization of the lobes were exposed.

Fairly distinct changes were noted in the kidneys of rats of group IV. Similar processes were observed, but were less distinct, in rats of group III, consisting in the swelling of the epithelium of convoluted tubules, and the appearance of protein and isolated hyaline casts in the lumina of the tubules.

On the basis of the data obtained in the course of exposure according to all the tests except the histopathological analysis, divinyl in concentrations of 1 and 3 mg/m^3 caused no visible changes in the condition of the animals; only a concentration of 30 mg/m^3, as indicated by the majority of the tests, produced substantial changes.

The presence of some morphologic changes in animals exposed to divinyl in 3 mg/m^3 concentration attests to the irritant effect of the gas on the mucous membranes of the respiratory tract and on the lungs and kidneys.

As a result, 1 mg/m^3 may be regarded as the inactive concentration of divinyl; this makes it possible to recommend it as the mean daily maximum permissible concentration.

In 1964, we studied the pollution of atmospheric air with divinyl around a plant producing synthetic rubber. The plant studied, the first section of which has been in use since 1963, produces synthetic divinyl-α-methylstyrene rubber. At the present time, the principal monomers, α-methylstyrene and divinyl, are produced at the plant.

Divinyl is prepared by dehydrogenating butane supplied by a petroleum refinery. Polymerization is carried out with a rosin emulsifier.

The sources of possible emission of divinyl into the atmosphere may be the sections of polymerization, charge preparation and distillation of monomers, and extraction of divinyl by chemical absorption from the cuprammonium complex, and the section where divinyl is stored.

The studies were carried out in July-August (170 samples) and in November-December 1964 (150 samples) at the temperature of the open air, from +25 to +30°C. in summer and from -7 to -15°C. in winter.

The samples were collected on a solid sorbent in U-shaped absorbers by means of water aspirators or pumps from a PKhR-54-MV instrument.

At only three points in summer did the divinyl concentration exceed the proposed highest single maximum permissible value (3 mg/m^3): at a distance of 50 and 70 m from the section of divinyl extraction (6.12 and 6 mg/m^3) and at 30 m from the section of distillation of monomers (3.31 mg/m^3).

In winter, a divinyl concentration in excess of 3 mg/m^3 was observed only at a distance of up to 20 m from these sections (3.2-3.5 mg/m^3).

At a distance of 500 m, the divinyl concentration was 0.2-0.7 mg/m^3; no divinyl was detected within a radius of 1500-2000 m.

The low boiling point of divinyl (-4°C.) and the high temperature of the ambient air in summer (from +20 to +30°C.) cause a considerable emission of the gas from the sections.

In the wintertime, the divinyl concentrations are lower at all the sampling points. It is, therefore, necessary to give particular attention to sealing all the systems in the plant sections, particularly in the summertime, so as to reduce the discharge of divinyl into atmospheric air.

Conclusions

1. On the basis of a study of thresholds of the effect of divinyl on the human organism (olfactory perception, light sensitivity of the eye and electrical activity of the brain) it is proposed that the highest single maximum permissible concentration of divinyl in atmospheric air be taken as 3 mg/m^3.

2. On the basis of a study of the resorptive effect of divinyl under prolonged round-the-clock conditions of exposure of animals, 1 mg/m^3 is proposed as the mean daily maximum permissible concentration of divinyl in atmospheric air.

3. As a method for determining microquantities of divinyl in atmospheric air in the presence of other products of synthetic rubber production, a spectrophotometric method is proposed for determining divinyl whereby samples of the latter are deposited on a solid sorbent by suction and extracted with isooctane and the resulting solution is analyzed on an SF-4 spectrophotometer.

LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1967 bibliography.

NEW EXPERIMENTAL DATA FOR THE VALIDATION OF THE MAXIMUM PERMISSIBLE CONCENTRATION OF HEXAMETHYLENEDIAMINE IN THE AIR OF POPULATED AREAS

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 10, Izdatel'stvo "Meditsina" Moskva, p. 15-32, (1967).

The Soviet and foreign literature on the problem of the biological effect of hexamethylenediamine is scarce. As far as the effect of hexamethylenediamine on the human organism in general, very scant data are available in this area. Such studies have been carried out in most cases on an insignificant number of experimental animals, and involved only high concentrations, the effect of hexamethylenediamine being appraised on the basis of very rough indicators.

Of greatest interest to us are literature data on the action of hexamethylenediamine entering the organism via the respiratory tract. Experimental studies of this action are given in only two papers by Soviet authors (V. S. Filatova, V. A. Russkikh, Ye. V. Chernet, and T. G. Lapina, 1951; A. P. Martynova, 1957).

The study of the biological action of hexamethylenediamine in acute and chronic experiments involving inhalational penetration into the organism showed that hexamethylenediamine is a toxic substance having a general resorptive effect manifested in an impairment of the permeability of fine and medium-sized blood vessels, various dystrophic changes in internal organs (lungs, liver, kidneys, spleen, heart muscle, brain and spinal cord, stomach and intestines). Hexamethylenediamine has a depressing effect on the central nervous system, decreases the arterial pressure, and changes the morphological composition of the blood. According to the data of A. P. Martynova (1957), hexamethylenediamine has such a toxic effect in concentrations of 1 mg/m^3 or higher; lower concentrations have not been studied.

As follows from a literature survey by Paton (1957), the mechanism of toxic action of hexamethylenediamine is based on the ability of mono- and diamines and certain other chemical substances to "liberate" bound histamine and possibly other biologically active substances. The greatest such effect is manifested by diamines, and also amines with a large number of carbon atoms.

On the basis of a study of the reflex effect of low-concentration hexamethylenediamine on the human organism (A. Ye. Kulakov, 1964), we proposed the highest single maximum permissible concentration of hexamethylenediamine in atmospheric air, 0.001 mg/m^3. The present paper reports on a round-the-clock three-month study of the effect of inhaling low hexamethylenediamine concentrations on the organism of experimental animals.

The study was made on 60 white male rats divided into four groups of 15 rats each. Group I of the animals was exposed to a hexamethylenediamine concentration of 1 mg/m^3 (the maximum permissible value for industrial buildings); group II, to 0.04 mg/m^3, and group III, to 0.001 mg/m^3 (highest single maximum permissible concentration in atmospheric air). The animals were observed during the period of exposure and also for one month before and after exposure by means of the following tests: weight dynamics of the animals, determination of the number of reticulocytes, leucocytes, and eosinophils in the blood, study of fluorescence* and phagocytic activity of the leucocytes; determination of the titer of Vi-antibodies following immunization with typhoid vaccine; study of the motor chronaxy of antagonist muscles, and analysis of the dynamics of excretion of coproporphyrin by the experimental animals. At the end of the exposure, histopathological studies of the internal organs were made.

The hexamethylenediamine concentrations in the exposure chambers were determined daily, and yielded the following data (Table 1).

Table 1

Average Concentrations of Hexamethylenediamine in the Exposure Chambers in Milligrams per Cubic meter

Group of Animals	M	σ	m	Total Analyses
I	1,086	0,25	0,018	129
II	0,043	0,0159	0,0014	118
III	0,0012	0,0005	0,00004	115

The weight of the animals was determined once every 10 days. It should be noted that the weight of animals of group I, even before exposure, lagged somewhat behind that of animals of the other groups (by an average of 5.7%). During the exposure, this lag increased, and after one month reached a statistically significant value that was preserved until the 80th day of exposure (Fig. 1).

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* Editor's note: For the Russian use of the terms "luminescent" and "luminescence" in this paper, we have substituted "fluorescent" and "fluorescence", on the basis of the definitions of these terms.

In groups II and III, no lag behind the weight of the control animals was observed. A. P. Martynova (1957) also noted a lag in the weight of the experimental animals when white rats were exposed to hexamethylenediamine in concentrations of 10 mg/m^3 and 1 mg/m^3 over the course of 6 months for 4 hours a day.

The number of reticulocytes was determined by A. P. Yegorov's method (1954). This study showed that only the action of hexamethylenediamine in a concentration of 1 mg/m^3 after two weeks of exposure caused a statistically significant increase in the number of reticulocytes (confidence factor, 99%) as compared with the control group; after the following two weeks, this increase returned to the original level (Fig. 2).

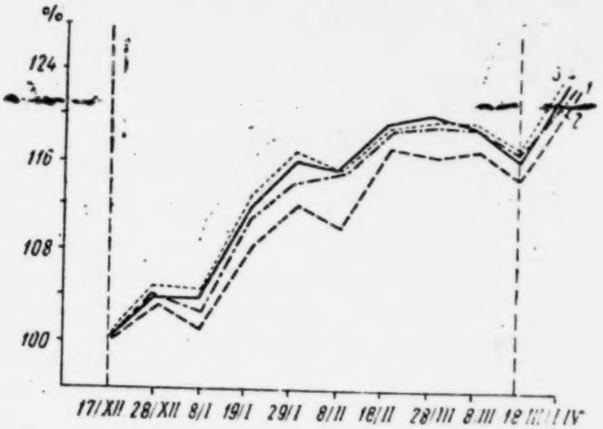

Fig. 1. Weight of experimental animals subjected to chronic inhalational exposure to low concentrations of hexamethylenediamine.
1 - concentration 1 mg/m^3; 2 - concentration 0.04 mg/m^3; 3 - concentration 0.001 mg/m^3; 4 - pure air

The number of leucocytes (method of N. M. Nikolayev, 1954) in the blood of white rats exposed to hexamethylenediamine did not differ appreciably from the data of the control group.

The content of eosinophils and other formed elements of the blood was found to depend on the functional state of the brain (V. N. Chernigovskaya and A. Ya. Yaroshevskiy, 1953, 1957; K. Kh. Kyrche, 1956, and others).

Many authors (Thorn, 1953; I. A. Eskin and G. M. Vidavskaya, 1956; Aschkenasy, 1957; I. A. Eskin and N. V. Mikhaylova, 1958, and others) showed that the content of eosinophils in peripheral blood is affected by the hormonal system anterior hypophyseal lobe - adrenal cortex, a system controlled by the hypothalamus. Recent studies in the area of occupational diseases show functional shifts in this hormonal system leading to a change in the content of eosinophils of the blood in man and also in experimental animals exposed to toxic substances in concentrations prevailing in the air of plant shops (Ye. I. Spynu, 1962; L. E. Gorn, 1963, and others).

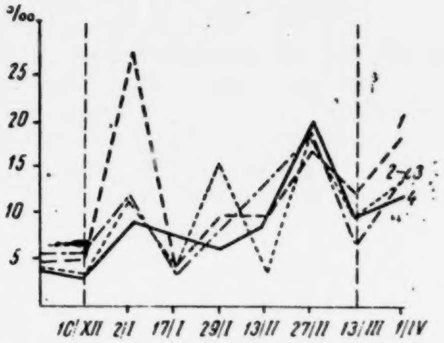

Fig. 2. Content of reticulocytes in the blood during chronic inhalational exposure to low hexamethylenediamine concentrations. Notation same as in Fig. 1.

As we know, the number of eosinophils in peripheral blood increases in an allergic state. Such a phenomenon is regarded as a defensive reaction, i.e., the ability of eosinophils to alleviate the stress of an allergic reaction by inactivating histamine, heparin and other biologically active substances whose levels in the free state increase sharply during the action of allergens (I. P. Lerner and Ye. S. Brusilovskiy, 1961; L. D. Grishpun, 1962, and others).

Of major interest is the elucidation of the effect of the hexamethylenediamine concentrations studied on the content of eosinophils in the peripheral blood of rats under experimental conditions.

The absolute quantity of eosinophils in peripheral blood was determined by the method of S. M. Bakman (1958) once a week during the first month of exposure and once every two weeks during the following month. A total of 223 blood analyses were performed.

Figure 3 shows that a decrease in the quantity of eosinophils of the blood was observed in all the groups of animals as early as one week following the start of exposure; in the second week, the most pronounced eosinopenic reaction occurred in animals of group I, but the difference from the control group still did not reach a statistically significant value. At the end of the first month, for technical reasons, the exposure was interrupted for two days (15 and 16 January), and 14-16 hours after the exposure was resumed, a sharp increase was observed in the number of eosinophils in the blood of animals of groups I and II as compared with the other groups. Particularly noticeable changes were observed in rat No. 3 in group I and rat No. 4 in group II. In one animals, the number of eosinophils increased from 474 to 1313, and in the second, from 200 to 837 in 1 mm^3 of blood. Subsequently, a new decrease in the number of eosinophils was observed in the animals of the first two groups which lasted until the end of exposure; the difference from the control group reached statistically significant values (95%).

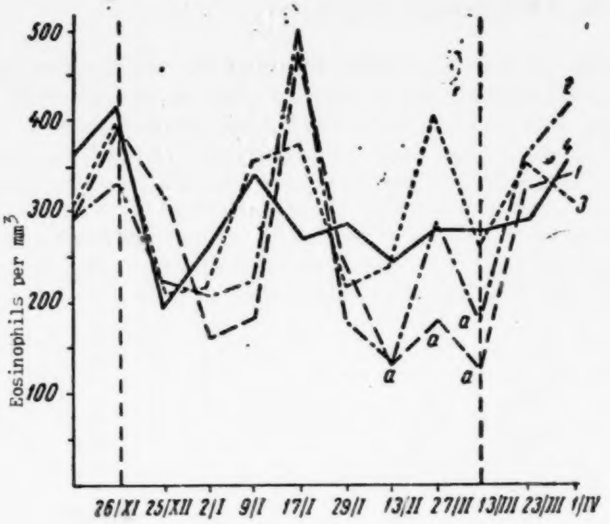

Fig. [illegible] Absolute quantity of eosinophils in the blood of experimental animals during chronic inhalational exposure to low hexamethylenediamine concentrations. Notation same as in Fig. 1.

In these groups, the content of eosinophils in the blood decreased by more than 50% as compared with the original data, which attested to the fact that the process involved compensatory mechanisms (S. M. Bakman, 1960, and others) accomplished by the hormonal system anterior hypophyseal lobe - adrenal cortex under the influence of hexamethylenediamine in concentrations of 1 and 0.04 mg/m^3.

The number of eosinophils in the blood of animals in group III (0.001 mg/m^3) was close to the data of the control group.

Thus, the studies show a nonspecific reaction of the organism to the adverse effect of microconcentrations of atmospheric pollutants. A number of authors (M. N. Meysel' and V. A. Sondak, 1956; S. S. Sergel' and A. A. Klimenko, 1957; M. Ya. Khodas, 1959; Ye. B. Zakrzhevskiy and L. G. Vasil'yeva, 1963, and others) showed that the nature of the fluorescence of leucocytes after vital staining with acridine orange changes in diseases of the blood or under the influence of ionizing radiation; the observed changes of the fluorescence of leucocytes from green to yellow, orange and red are due to physicochemical changes in the cell.

During the inhalational action of low concentrations of aniline on the organism, a change in the fluorescence of leucocytes was noted by A. D. Semenenko (1963) and P. G. Tkachev (1963), and in the case of amylenes, by M. I. Gusev and K. N. Chelikanov (1963).

The dye used was acridine orange diluted in the proportion of 1:50,000, prepared in saline solution. The blood in the amount of 0.02 ml was taken from the rats' tails and placed in a test tube containing 0.18 ml of the acridine orange solution. One drop of fluorochromed blood was then taken from the test tube, transferred onto a glass slide, and covered with a cover glass. The specimen was immediately examined with a Reichert microscope under a magnification of 5 x 60. One hundred leucocytes were counted in each specimen, and the percentage of leucocytes with altered fluorescence was calculated. A total of 182 blood analyses were made.

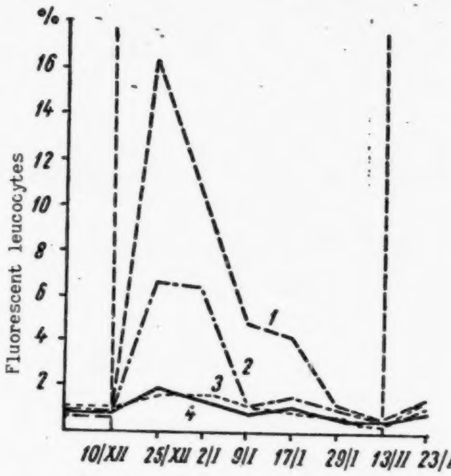

Fig. 4. Content of leucocytes with altered fluorescence (in percent) for inhalational chronic exposure to low hexamethylenediamine concentrations. Notation same as in Fig. 1.

It is evident from Fig. 4 that in animals of group I, the percentage of leucocytes with altered fluorescence increased sharply after a week of exposure (in some animals up to 21-25%) and returned to the original level at the start of the second month of exposure. In animals of group II, this increase was less pronounced (up to 9-11% in some animals) and returned to the original level after 3 weeks of exposure. The restoration of altered fluorescence of leucocytes over the course of the exposure of the rats to low concentrations of amylenes was also observed by M. I. Gusev and K. N. Chelikanov (1963). The increase in the percent content of leucocytes with altered fluorescence in animals of groups I and II as compared with the control group is characterized by statistically significant values (95 and 99%). There were also observed mostly yellow and orange fluorescent tones of the leucocytes and only in isolated cases, red ones. In the case of animals of group III, the data obtained for them differed little from the control group.

As we know, the defensive-adaptive function of the organism is related to the general physiological responsiveness of the organism. The latter in turn depends on numerous environmental factors (physical, chemical, biological, alimentary, etc.) which, by acting on the organism, can change its responsiveness by either reinforcing or weakening it (A. D. Ado).

Since the immunobiological responsiveness of the organism is a partial manifestation of its general biological responsiveness, this immunobiological responsiveness can be used to evaluate that of the specific and general resistive power of the organism.

Recent studies in the area of occupational diseases (T. V. Rasskazova, 1958; A. P. Volkova, 1959; V. K. Navrotskiy, 1960; A. I. Pakhomychev, 1960; A. F. Stoyanovskiy, 1961, and others) indicate a change in the state of the immunobiological responsiveness of the organism under industrial conditions, and also in experiments on animals subjected to a prolonged inhalational action of a number of noxious substances (sulfur dioxide, carbon monoxide, lead, tetraethyllead, carbon tetrachloride, nitrobenzene, gasoline, aniline, etc.). In these studies, the authors noted a depression in the phagocytic activity of leucocytes and also in the formation of antibodies during exposure to noxious substances in concentrations exceeding or equal to the level of the maximum permissible values for air in industrial buildings.

In connection with the validation of the maximum permissible concentration of hexamethylenediamine in atmospheric air, we deemed it interesting to determine whether a decrease occurred in the immunobiological indicators during chronic exposure to microconcentrations of hexamethylenediamine.

As the object of phagocytosis in our study we selected a one-day culture of staphylococcus in a concentration of 500 million microbial bodies per ml based on the optical standard.

The blood, a 2% solution of sodium citrate, and the microbial suspension were taken in equal volumes (0.06 ml). The phagocytosis reaction lasted 20 minutes at 37°. After centrifuging, the film of leucocytes was removed and used to prepare smears, which were stained by the Gimze-Romanovskiy method.

In the specimen, 100 neutrophiles were counted and the percentage of active leucocytes (extent of phagocytosis) was calculated, along with the average content of microbial cells in a single leucocyte which had phagocytized (intensity of phagocytosis) (V. S. Gostev et al., 1950). To determine the phagocytic activity of leucocytes, 204 blood analyses were carried out; the first two were separated by a one-week interval and subsequent determinations were made every two weeks.

Results of the analysis showed (Fig. 5) that the percentage of active leucocytes in animals of group I (1 mg/m^3) decreases sharply after only one week of exposure to hexamethylenediamine, and after two weeks it increases and even slightly exceeds the indices of the control group. Subsequently, a new depression of leucocyte activity takes place which is preserved until the end of exposure.

The most pronounced change in leucocyte activity was observed in animal No. 14 of group I. Before the start of exposure, the percentage of active leucocytes in this animal was 99.3-100, and one week after the start of exposure it decreased to 55.1. After two weeks, the percentage of active leucocytes increased to 92.9, then decreased again to 79.3, without reaching the original level during the exposure. After the exposure was completed, the percentage of active leucocytes in animals of this group rapidly reached the background level. The same type of statistically significant but less pronounced changes were observed in animals of group II (0.04 mg/m^3).

It is evident from Fig. 6 that similar and significant changes also take place in the intensity of the phagocytic process in animals of groups I and II. In this case, the most pronounced change in the intensity of the process is also observed in animal No. 14 of group I. Before the start of exposure, the intensity index of phagocytosis was 7.4-7.9, and a week later it decreased to 1.3. After two weeks, the index increased to 5.3, then decreased again to 2.5 and remained at this level with some fluctuations until the end of exposure.

The dynamics of the phagocytic activity of leucocytes in animals of group III (0.001 mg/m^3) did not differ substantially from the data for the control group.

It should be noted that the dynamics of the phagocytic process according to both indices in all the animals of groups I and II had a general pattern which was chiefly manifested in a depression of the process. An increase in phagocytic activity in the second week of exposure after its sharp depression indicates phase changes of the phagocytic process during chronic inhalational exposure of the rats to hexamethylenediamine in concentrations of 1 and 0.04 mg/m^3.

The experimental animals were vaccinated with typhus Vi vaccine prepared at the Moscow Scientific Research Institute of Epidemiology and Microbiology.

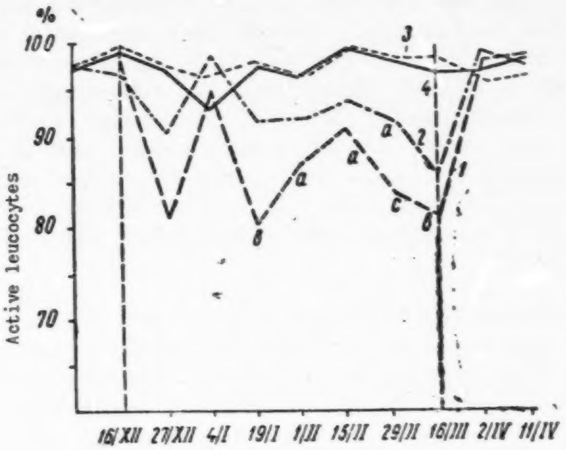

Fig. 5. Phagocytic activity of leucocytes (based on the extensive index) in experimental animals during chronic inhalational exposure to low hexamethylenediamine concentrations. Notation same as in fig. 1; Degree of significance: a - 95%; b - 99%; c - 99.9%.

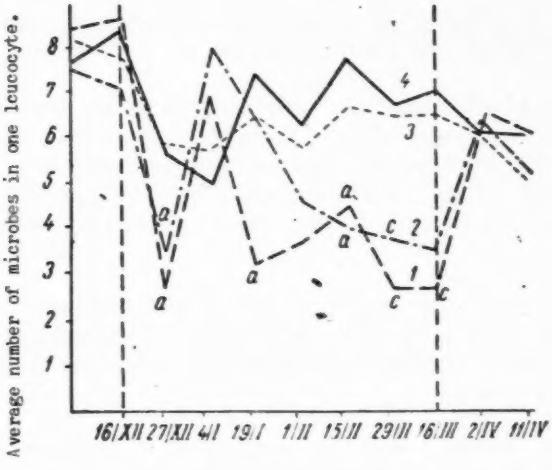

Fig. 6. Phagocytic activity of leucocytes (based on the intensive index) in experimental animals during chronic inhalational exposure to low hexamethylenediamine concentrations.

Notation same as in Figs. 1 and 5.

In the literature, we were unable to find the procedure for immunizing rats with this vaccine, and therefore the study was somewhat exploratory in character. The vaccination was started after one month of exposure to hexamethylenediamine, when a change in the responsiveness of the organism could be expected. The vaccine was administered by hypodermic injection into the hind leg. In the first vaccination, 200 million microbial bodies of the typhus bacillus were introduced along with 0.04 mg of typhus Vi antigen; the second vaccination (one week later) and the subsequent ones contained 300 million microbial bodies and 0.06 mg of Vi antigen each. Blood in the amount of 0.5-0.6 ml was taken from the rats' tails, and after being allowed to stand at room temperature for 24 hours, the serum was drawn off, then stored at 34°. The level of the titer of Vi antibodies in the serum was determined by means of the passive hemagglutination reaction (N. A. Kraskin and N. M. Gutorova, 1962).

Before the administration of the vaccine, the level of the titers of Vi antibodies in the experimental animals was equal to zero. One week after the vaccination, the formation of Vi antibodies was noted in the animals. However, the level of the titer of Vi antibodies in animals of group I clearly lagged behind the animals of the control group (by a factor of 2) and no Vi antibodies whatsoever were found in the blood serum of animal No. 12 in group I. A lag in antibody production was also observed after the second administration of vaccine (by a factor of 2.3), and the level of decrease of the titer in animals of this group surpassed the decrease of the titer in animals of the control group.

The same kind of effect, although less extensive, was also observed in the dynamics of production of Vi antibodies in animals of group II.

A statistically significant difference (95%) in the level of the titer of Vi antibodies as compared with the control group was observed only in animals of group I one week after the second vaccination. The level of the titer of Vi antibodies after vaccinations performed at the end of exposure and during the recovery period did not differ substantially from data for the control group.

In animals of group III, which received the lowest concentration of hexamethylenediamine (0.001 mg/m^3), the level of production of Vi antibodies did not differ from data of the control group over the entire course of the exposure.

We also studied the dynamics of excretion of coproporphyrin with the urine by determining it on an SF-4 spectrophotometer (M. I. Gusev and Yu. I. Smirnov, 1960). The daily urine was collected in special receivers every 10 days from five rats of each group. Three urinalyses each were carried out before the exposure and during the recovery period, and 9 urinalyses in each group were performed in the course of exposure. The fluctuations of coproporphyrin in the urine of animals of group I which we observed during the period of exposure indicated a certain tendency toward a decrease of its excretion on some days of the analysis during the first half of exposure, but this difference was not confirmed statistically. The significant difference observed during the recovery period was due primarily to a decrease in the content of coproporphyrin in the urine of animals of the control group. No substantial differences from the control group were observed in groups II and III.

One of the tests used during the experiment was the determination of the ratio of chronaxies of antagonist muscles in the experimental animals, performed every 10 days with the aid of an ISE-01 pulse stimulator. Results of the tests showed that a disturbance of the normal ratio of the motor chronaxy of antagonist muscles occurred in animals of groups I and II after one month of exposure and remained at this level with certain fluctuations during the next 40 days of exposure (Fig. 7); the normal ratio of the motor chronaxy was subsequently restored. A statistically significant difference in the animals of groups I and II as compared with the control was observed on the 50th day of exposure (degree of significance 99% in both groups). The dynamics of the ratio of the motor chronaxy of antagonist muscles in animals of group III (0.001 mg/m^3) was within the range of physiological fluctuations.

The pathologico-anatomic study* was made on 45 white rats, 26 of which were sacrificed immediately after the exposure, and 19 after two months in order to study the distant results of the exposure.

Autopsy of animals of group I (1 mg/m^3) revealed a depletion of the subcutaneous adipose cellular tissue. Histopathological analysis of the liver of animals of this group showed venous hyperemia (Fig. 8), impregnation of the vessel walls with plasma, and perivascular lymphoid-histiocytic nodules. The glycogen content of the liver was stable, with a slight decrease in some cases.

In the myocardium, against a background of venous hyperemia, there were individual fibers or groups of fibers with uneven staining, with a distinct transverse striation of these fibers and of the nucleus. In the kidneys, only hyperemia and lymphoid infiltration along the course of the tubules were observed. In the adrenal glands, lipids were found in all three zones.

In the brain of animals of group I, the capillary network was moderately enlarged. The cerebral cortex and subcortical ganglia showed the presence of cells with a vacuolated cytoplasm, diffusion or coarsening of the granules of Nissl substance, seldom with chromatolysis. Cells with pericellular edema were also found (Fig. 9).

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* The pathologico-anatomic studies were conducted at the pathologic anatomy laboratory of the A. N. Sysin Institute of General and Communal Hygiene of the USSR Academy of Medical Sciences under the supervision of Doctor of Medical Sciences V. P. Osintseva.

In animals of group II (0.04 mg/m^3), the liver, myocardium, kidneys, and brain showed the same changes as in animals of group I, but the changes were less distinct. In the adrenals, the amount of lipids had a tendency to decrease.

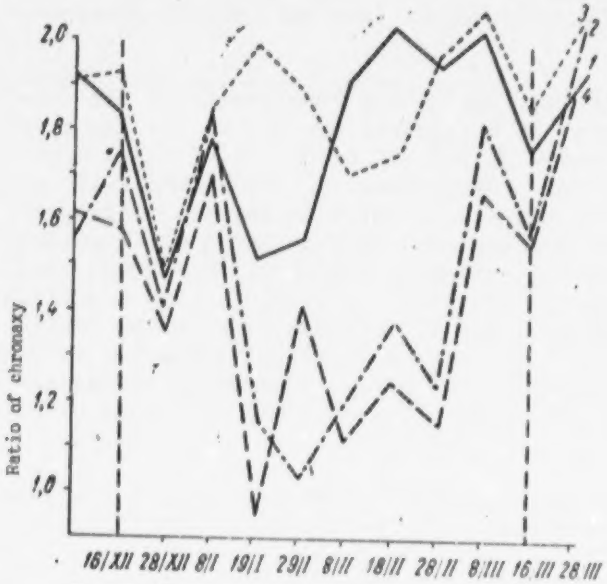

Fig. 7. Ratio of motor chronaxy of antagonist muscles in experimental animals during chronic inhalational exposure to low hexamethylenediamine concentrations. Notation same as in Fig. 1.

A study conducted two months after the exposure showed that purulent abscessing pneumonias were observed more often in animals of groups I and II than in other groups, and the gravity and extent of the process increased with rising concentration of hexamethylenediamine.

Changes in the organs of animals exposed to hexamethylenediamine in concentrations of 1 and 0.04 mg/m^3 were of the same type in both the immunized and non-immunized rats. The spleen of immunized animals showed follicles more frequently than that of nonimmunized ones, and their size was much larger. In the immunized animals, the number of plasma cells, to which the formation of antibodies was attributed, increased substantially. The morphology of the immune process in rats of groups I and II (1 and 0.04 mg/m^3) was somewhat less marked than in animals of the other groups.

In animals exposed to hexamethylenediamine in a concentration of 0.001 mg/m^3 there were no morphological changes that differed from the control group.

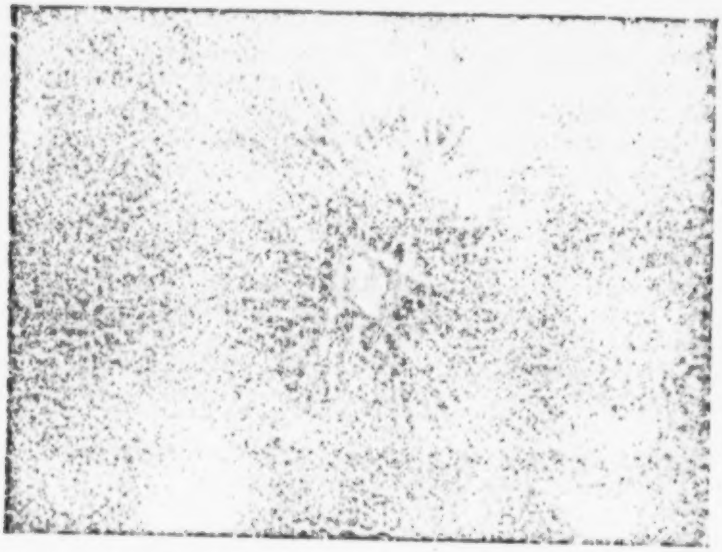

Fig. 8. Liver of animal No. 2 of group I. Hematoxylin-eosin staining. Magnification 10 x 10.

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Fig. 9. Brain of animal No. 1 of group I. Nissl staining. Magnification 90 x 15.

Thus, the results of our studies showed the adverse effect on the organism of low hexamethylenediamine concentrations (1.0 and 0.04 mg/m^3), manifested chiefly in a certain lag in the weight of animals of group I and an increase in the number of reticulocytes. In the blood of groups I and II of the experimental animals, we also observed an eosinopenic reaction and an increase in the percent content of leucocytes with an altered fluorescence.

From the above data it also follows that such low concentrations of hexamethylenediamine are capable of altering the state of immunobiological responsiveness of the organism, causing a depression of the processes of phagocytosis, antibody formation, and morphology of the immune process. Groups I and II also showed a disturbance of the normal ratio of chronaxy of antagonist muscles and pathologico-anatomic changes in the internal organs.

Only a hexamethylenediamine concentration of 0.001 mg/m^3 was found to be inactive according to all the tests performed.

Conclusions

1. Hexamethylenediamine in a concentration of 1 mg/m^3 during inhalational round-the-clock exposure for three months has an adverse effect on the organism of the experimental animals (slight weight lag, increase in the number of reticulocytes, eosinopenia, increase in the content of leucocytes with altered fluorescence, depression of the phagocytic activity of leucocytes, production of Vi-antibodies, immunomorphological reactions following artificial immunization, disturbance of the normal ratio of the motor chronaxy of antagonist muscles, pathologico-anatomic changes in internal organs).

The same changes, but less evident, were caused by hexamethylenediamine in a concentration of 0.04 mg/m^3 (with the exception of the weight dynamics of the animals and number of reticulocytes).

2. Hexamethylenediamine in a concentration of 0.001 mg/m^3 under the same conditions of exposure for three months had no effect on the organism of the experimental animals.

3. The mean daily maximum permissible concentration of hexamethylenediamine in the atmospheric air of populated areas can be adopted at the level of 0.001 mg/m^3, which we propose as the highest single concentration.

LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1967 bibliography.

LITERATURE CITED IN 1968 PAPERS

Алексеева М. В. Определение атмосферных загрязнений. М., Медгиз, 1963.

Алексеева М. В. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 8. М., 1964, стр. 174.

Алексеева М. В., **Андронов** Б. Е., **Гурвиц** С. С., **Житкова** А. С. Определение вредных веществ в воздухе производственных помещений. М., Госхимиздат, 1954.

Алексеев Н. Г. В кн.: Руководство по клиническим и лабораторным исследованиям, основанное В. Е. Предтеченским. М., 1964, стр. 33.

Альперн Д. Е. В кн.: Современные вопросы нервизма в физиологии и патологии. М., 1958, стр. 41—46.

Аничков С. В. Фармакол. и токсикол., 1955, XVIII, 1, 3—7.

Ахмеров У. Ш. Журн. высш. нерв. деят., 1956, 6, 2, 226—233.

Байков Б. К. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 8. М., 1964, стр. 127—137.

Бакман С. М. Лабор. дело, 1958, 5, 13—15.

Беленький М. Л., **Розенгарт** В. И. Успехи совр. биол., 1949, 28, 3 (6), 387—399.

Белозерский А. Н. Вестник МГУ, 1949, 2, 125.

Бирюкова Р. Н. Гиг. и сан., 1962, 7, 43—46.

Буштуева К. А. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 4. М., 1960, стр. 92.

Буштуева К. А. Гигиеническая оценка окислов серы как атмосферных загрязнений (экспериментальные исследования). Автореф. дисс. докт. М., 1964.

Буштуева К. А. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 8. М., 1964, стр. 119—137.

Буштуева К. А., **Полежаев** Е. Ф., **Семененко** А. Д. Гиг. и сан., 1960, 1, 57—61.

Бухтияров А. Г. В кн.: Механизмы патологических реакций. Л., 1955, стр. 183—187.

Валиев Ю. Ф. Лабор. дело, 1964, 7, 434—438.

Волкова А. П. Гиг. и сан., 1959, 1, 80—82.

Волкова А. П. Изменение фагоцитарной реакции организма при хроническом воздействии бензола (в производственных условиях и эксперименте). Автореф. дисс. канд. М., 1961.

Воробьева Р. М., **Суворов** С. В. Гиг. труда и проф. заболевания, 1961, 10, 45—48.

Гадаскина И. Д., Штессель Т. А. Физиол. журн. СССР, 1935, XIX, 6.

Гельденскнольд Р. С. В кн.: Информационный бюллетень Института им. Ф. Ф. Эрисмана, 1958, 14—15, стр. 50—53.

Гендерсон и Хаггард. Вредные газы в промышленности (перевод с немецкого). М.—Л., 1930.

Гершанович З. С., Минкина А. И. Биохимия, 1951, 16, 1, 36—39.

Гершуни Г. В. В кн.: Материалы по физиологии рецепторов. М., 1948, стр. 23—30.

Голубев А. А., Русин В. Я. В кн.: Руководство по гигиене труда. М., 1963, стр. 109—121.

Гольдблатт М. В., Гольдблатт Ю. В кн.: Некоторые проблемы гигиены труда и профессиональной патологии. М., 1960, стр. 70—77.

Горшков С. И. Скрытое время рефлекторных реакций как адекватный показатель функционального состояния нервной системы. Автореф. дисс. докт. М., 1963.

Гофмеклер В. А. Гиг. и сан., 1960, 4, 9—14.

Гофмеклер В. А. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 5, М., 1961, стр. 142—168.

Гурвич А. Е. Лабор. дело, 1955, 3, 3—9.

Гуринов Б. П. В кн.: Охрана труда и здоровья нефтяников Башкирии. М., 1958, стр. 139—148.

Гусев И. С. Гиг. и сан., 1965, 12, 6--11.

Гусев М. И. Гигиеническая оценка загрязнения атмосферного воздуха свинцом. Автореф. дисс. М., 1960.

Гусев М. И., Смирнов Ю. К. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 4. М., 1960, стр. 139—142.

Данишевский С. Л. В кн.: Вопросы гигиены труда и профзаболеваний. М.—Л., 1951, т. 10, стр. 187.

Данишевский С. Л. Фармакол. и токсикол., 1950, № 3, 16.

Двоскин Я. Г. Гиг. и сан., 1961, 10, 41—47.

Дебов С. С. В кн.: Успехи биологической химии. Под ред. В. Н. Ореховича. Т. 2. М., 1954, стр. 115

Дементьева М. И. В кн.: Анализ производственных газов. Л.—М., 1959.

Деянова Е. В. В кн.: Инструктивные материалы по методам определения в воздухе рабочих помещений некоторых химических веществ. М., 1964.

Добровольский Л. А. Гиг. труда и проф. заболевания, 1962, 2, 19—26.

Добрякова О. А. Проблемы физиологии оптики. М., т. 2, 1964.

Дубровская Ф. И. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 3. М., 1957, стр. 44—62.

Дюжева Ю. В. В кн.: Ученые записки Московского научно-исследовательского института гигиены имени Ф. Ф. Эрисмана. В. 5. М., 1960.

Елфимова Е., Пушкина Н. Н. Гиг. и сан., 1966, 2, 85—86.

Жилова Н. А. Гиг. и сан., 1959, 12, 18—23.

Закутинский Д. И., Парфенов Ю. Д., Селиванова Л. Н. Справочник по токсикологии радиоактивных изотопов. Медгиз, 1962.

Здродовский П. Ф. Вестн. АМН СССР, 1964, 9, 86—91.

Здродовский П. Ф. Вестн. АМН СССР, 1961, 4, 9—19.

Ибрагимов М. Г., **Лиснянский** Е. З. В кн.: Труды отоларингологов Башкирской АССР. В. 3. Уфа, 1962, стр. 16—22.

Имашева Н. Б. Гиг. и сан., 1963, 2, 3—8.

Ишимова Л. М. В кн.: XV научная сессия Медицинского института. Тезисы докладов. Куйбышев, 1954, 57—58.

Ицкович А. А., **Виноградова** В. А. В кн.: Тезисы докладов научной сессии Новосибирского санитарного института. Новосибирск, 1956, стр. 23—26.

Калюжный Д. Н., **Гордыня** М. П. В кн.: Материалы Всесоюзной конференции по санитарной охране атмосферного воздуха. М., 1964, стр. 54—57.

Каменский С. С. Материалы для фармакологии ацетофенона. Дисс. докт. СПБ, 1878.

Капланский С. Я. Вестн. АМН СССР, 1962, 9, 10—21.

Кнабенгоф В. Г. Гиг. труда и тех. безоп., 1934, 4, 22—26.

Корбакова А. И., **Кремнева** С. Н., **Кулагина** Н. К., **Уланова** И. П. В кн.: Промышленная токсикология. М., 1960, стр. 232—238.

Корнеев Ю. Е. Гиг. и сан., 1965, 9, 15.

Коштоянц Х. С. Юбилейный сборник АН СССР. М., 1947, 437—464.

Кравков С. В. Глаз и его работа. М.—Л., 1950.

Куприц Е. Н. Гиг. труда и проф. заболевания, 1925, 1, 80—81.

Лавров Б. А., **Яновская** Б. И. В кн.: Витамины. Т. 2. Киев, 1956, стр. 61—64.

Лазарев Н. В. В кн.: Вредные вещества в промышленности. Ч. I. Госхимиздат. Л., 1954, стр. 112—131, 323.

Лазарев Н. В. В кн.: Вредные вещества в промышленности. Ч. I—II. Л., 1963.

Ли Шэн. Гиг. и сан., 1961, 8, 11.

Любимова М. П. Сборник экспериментальных исследований по промышленным ядам. В. XXV. Л., 1936, стр. 29—36.

Манинова Х. Х. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 8. М., 1964, стр. 145—160.

Мерков А. М. Общая теория и методика санитарно-статистического исследования. М., 1960.

Миллер С. В. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 2. М., 1955, стр. 29—46.

Митина Л. С. Гиг. и сан., 1962, 10, 3—8.

Михайлец А. Г., **Пельц** Д. Г. В сб.: Токсикология сероорганических соединений. Уфа, 1964, стр. 4—20.

Михельсон М. Я. Действие наркотиков на холинэстеразу. Л., 1948.

Мухитов Б. М. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 7. М., 1963, стр. 76—98.

Мытник П., **Генкин** С. В кн.: Труды XI съезда терапевтов СССР. Госмедгиз, 1932, стр. 253.

Назыров Г. Н. Медицинский журнал Узбекистана, 1960, 8, 28—32.

Нейман Ю. В., **Смолин** В. В. В кн.: Труды Всесоюзного совещания по защите от пыли и газов. М., 1964.

Новиков Ю. В. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 3. М., 1957, стр. 85—107.

Ольшанский М. И., **Лихачева** В. В. Труды Ростовского-на-Дону медицинского института. Сб. 2, Ростов-на-Дону, 1935, стр. 120.
Павлов И. П. Лекции по физиологии. Изд. АН СССР, Л.—М., 1952.
Опарин А. И., **Евреинова** Т. Н. Вестн. АН СССР, 1917, 58, 253.
Петров А. М. В кн.: Труды Ленинградского сан.-гиг. мед. института. Л., 1960, стр. 153.
Погосян У. Г. Гиг. и сан., 1965, 7, 3.
Покровский А. А. Воен. мед. журн., 1953, 9, 61—65.
Преображенский Б. С. Вестн. АМН СССР, 1964, 10, 7—16.
Пушкина Н. Н. Биохимические методы исследования. М., Медгиз, 1963.
Пьянков В. В. Журн. общей химии, 1933, 3, 6.
Пыцкий В. И. Вестн. АМН СССР, 1963, 4, 20—28.
Ревнова Н. В. Гиг. труда и проф. заболевания, 1965, 2, 17—22.
Розенбаум Н. Д. Гиг. и сан., 1945, 1—2, 23—29.
Розенцвит Г. Э. В кн.: Рефераты научных работ Ленинградского института гигиены труда за 1953 г. Л., 1954, стр. 148—152.
Рязанов В. А. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. I. М., 1956, стр. 26—39.
Рязанов В. А. Руководство по коммунальной гигиене. Т. I. М., 1961.
Рязанов В. А. Гиг. и сан., 1961, 6, 3—8.
Рязанов В. А. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 8. М., 1964, стр. 5—21.
Рязанов В. А., **Буштуева** К. А., **Новиков** Ю. В. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 3. М., 1957, стр. 26—39.
Садилова М. С., **Селянкина** К. П., **Штуркина** О. К. Гиг. и сан., 1965, 5, 11—15.
Самарская Н. В. В кн.: Материалы XIII научной сессии Свердловского института гигиены труда и профпатологии. Свердловск, 1964.
Селина И. А. Гиг. и сан., 1962, 5, 41.
Семененко А. Д. Гиг. и сан., 1963, 7, 49—55.
Семененко А. Д., **Балашев** Б. Н., **Арзамасцев** Е. В. В кн.: Всесоюзная конференция по вопросам гигиены воды и санитарной охраны водоемов. Тезисы докладов. М., 1963, стр. 33—35.
Симаков П. В. Вопр. питания, 1960, 19, 6, 69—71.
Смирнов Ю. К. Нарушения порфиринового обмена при заболеваниях нервной системы. Автореф. дисс. М., 1953.
Смоличев Е. П. Арх. пат., 1961, 23, 5, 59—64.
Соломин Г. И. Гиг. и сан., 1961, 5, 3—8.
Спирин А. С. Биохимия, 1958, 23, 5, 656.
Титаев А. А., **Ларский** Э. Г., **Борисова** Т. П., **Надеждина** Е. А. Лабор. дело, 1964, 4, 201—205.
Ткач Н. З. Гиг. и сан., 1965, 8, 5.
Ткачев П. Г. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 8. М., 1964, стр. 41—58.
Убайдуллаев Р. Гиг. и сан., 1961, 7, 3—11.
Убайдуллаев Р. Лабор. дело, 1964, 5, 300—302.
Уфлянд Ю. М. Теория и практика хронаксиметрирования. Л., 1941.
Учитель И. Я., **Хасман** Э. Л. Вестн. АМН СССР, 1964, 3, 23—36.
Филатова В. Н. Гиг. и сан., 1962, 11, 4.

Филиппова З. Х. В кн.: Материалы научной конференции, посвященной вопросам гигиены труда, профпатологии и промышленной токсикологии в нефтяной и нефтехимической промышленности. Уфа, 1961, стр. 170—177.

Флюрр Ф., Церник Ф. Вредные газы. М., 1938.

Фролова И. Н. В кн.: Промышленная токсикология и клиника профессиональных заболеваний химической этиологии. М., 1962, стр. 186—188.

Черниговский В. Н. Физиол. журн. СССР, XXXIII, 1, 1947, 17—27.

Чижиков В. А. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 8. М., 1964, стр. 21—40.

Шибков А. И. К учению об отравлении уксусной кислотой или ее эссенцией. Казань, 1915.

Шлейфман Ф. М. Гиг. и сан., 1961, 5, 14—18.

Штессель Т. А. Фармакол. и токсикол., 1941, 4, 3, 55—59.

Шульга Т. М. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. 6. М., 1962, стр. 128—145.

Якамээс В. А. Гиг. и сан., 1966, 3, 3—7.

Ярославская Р. И. Действие бензола в эксперименте на некоторые функции центральной нервной системы. Автореф. дисс. канд. Харьков, 1952.

Campbell R. M., Kosterlitz H. W. Brit. J. exp. Path., 1952, 29, 149.

Chrisfomanos A. Biochem. J., 1930, 229, 248.

Deichmann V. B., McDonald W. E., Bernal E. Toxicol. a. Appl. Pharmacol., 1963, 5, 2.

Dujardin, Beaumetz, Bardet. Bull. gen. Therap., 1887, Janv. 15, 1.

Ehrich V., Drabkin D., Forman G. J. exp. Med., 1949, 90, 157.

Ghiringhelli Di Fabio Med. laboro, 1957, 5 48, 10, 559.

Goret V. P., Joubert L. Rev. Immunol. (Paris), 1949, 13, 6, 338.

Huchard. Jaz. hebd., 1885, 50, 846.

Hultgren J. C. R. Soc. Biol., 1926, 95, 1068—1070.

Iamado Arch. Hyg., 1908, 61, 57.

Kuhböck J., Lachnit V. Internat. Arch. Gewerbepathol. u. Gewerbehyg., 1962, 19, 2, 149—157.

Kutscher W., Wust H. Biochem. Z., 1942, 310, 292—301.

Labordé C. R. Soc. Biol., 1835, 12, 19.

Mairet, Combemale. C. R. Soc. Biol., 1885, 11, 3, 178.

Massart L., Dufait R. Naturwissenschaften, 1939, 27, 806—807.

McCord C. R. Benzol (Benzene) poisoning Cincinnati. Ohio, 1931, p. 43.

Rambach V. A., Moomav D. R., Aet H. L., Cooper I. A. Proc. Soc. exp. Biol. (N. Y.), 1952, 79, 59.

Reiner J. M., Tsuboi K. M., Hudson P. B. Arch. biochem. biophys., 56, 165—183.

Rurt N. S., Murray R. G., Rossiter R. L. Blood, 1951, 6, 906.

Smith Q., Singer L., Armstrong W. D. J. Dent. Res., 1954 33, 685.

Wooster H. A., Lushbaugh C. C., Redemann C. E. J. Industr., Hyg. Toxicol., 1947, 29, 1, 56.

LITERATURE CITED IN 1967 PAPERS

а) Отечественная

Агеева-Майкова О. Г. Вестн. оторинолар., 1951, 3, 3—8.

Айзенберг М. Д. Русская отоларингология., 1927, 5, 494—502.

Аксюк А. Ф., **Булычев** Г. В. Гиг. и сан., 12, 1962.

Алексеева М. В. Определение атмосферных загрязнений, 1959.

Алексеева М. В. Определение атмосферных загрязнений, 1962.

Алексеева М. В. Предельно допустимые концентрации атмосферных загрязнений, 1964, в. 8, стр. 174—176.

Алпатов И. М. Гиг. труда и проф. заболевания, 1964, 2, 14—18.

Альперн Д. Е. В кн.: Современные вопросы нервизма в физиологии и патологии. М., 1958, стр. 41—46.

Альперн Д. Е. Холинергические процессы в патологии. М., 1963.

Анатовская В. С. Тезисы докладов. Материалы научной сессии по токсикологии высокомолекулярных соединений. М.—Л., 1961, стр. 28—29.

Анин Ю. Л. Лабор. дело, 1964, 1, 19—21.

Аничков С. В. Гиг. и сан., 1952, 10, 7—12.

Анохин П. К. Проблема центра и периферии в физиологии нервной деятельности. Горький, 1935, стр. 162.

Асс Т. В., **Вол** В. В. и др. Труды и материалы Украинского государственного института рабочей медицины, 1926, стр. 11—55.

Байков Б. К. Гиг. и сан., 1953, стр. 3—8.

Байков Б. К. В кн.: Предельно допустимые концентрации атмосферных загрязнений, 1964, в. 8, стр. 127—138.

Бакалейник К. Е. В кн.: Тезисы 10-й научной сессии Свердловского института гигиены труда и профессиональной патологии. Свердловск, 1960, стр. 59—60.

Бакман С. М. Лабор. дело, 1958, 5, 13—15.

Бакман С. М. Врач. дело, 1960, 11, 110—105.

Балаховский С. Д. и **Балаховский** И. С. Методы химического анализа крови. М., 1953, стр. 723.

Беленький М. Л. Элементы количественной оценки фармакологического эффекта. Изд. АН Латвийской ССР. Рига, 1959.

Бесков С. Д. Основы химической технологии. М., 1962.

Бехтерева Н. П. и **Усов** В. В. Физиол. журн. СССР, 1960, 46, 1, 108—111.

Бонград Э. М. и **Шляпин** В. Ф. Гиг. труда и проф. заболевания, 1960, 2, 9.

Бонград Э. М. и **Шляпин** В. Ф. Тезисы докладов научной конференции Горьковского института гигиены труда и профзаболеваний, посвященные итогам научно-исследовательской работы института за 1957 г. Горький, 1958, стр. 20.

Борисенкова Р. В. Экспериментальные исследования по гигиенической характеристике производственной пыли ферросплавов. Дисс. канд. М., 1952.

Борисова М. К. В кн.: Предельно допустимые концентрации атмосферных загрязнений, 1960, в. 4, стр. 61—75.

Буштуева К. А. Гигиеническая оценка окислов серы как атмосферных загрязнений (экспериментальные исследования). Автореферат, 1964.

Буштуева К. А. В кн.: Предельно допустимые концентрации атмосферных загрязнений. М., 1961, стр. 118—126.

Буштуева К. А. В кн.: Предельно допустимые концентрации атмосферных загрязнений. М., 1960, в. 4, стр. 92—101.

Буштуева К. А. В кн.: Предельно допустимые концентрации атмосферных загрязнений. М., 1962, в. VI.

Буштуева К. А. В кн.: Предельно допустимые концентрации атмосферных загрязнений. 1957, в. 3, стр. 23—41.

Буштуева К. А., Полежаев Е. Ф., Семененко А. Д. Гиг. и сан., 1960, 1, 57—61.

Быховская С. М., Гинзбург С. А., Хализова О. Д. Методы определения вредных веществ в воздухе и других средах. Ч. I. 1960, стр. 104—107.

Вацулик П. Химия мономеров. Т. I. Пер. с чешского. Под ред. акад. И. Л. Кнунянца. Изд. Иностранной литературы. М., 1960.

Величко Ф. К. В кн.: Краткая химическая энциклопедия. М., 1961, т. I, стр. 90—91.

Войнар А. О. Биологическая роль микроэлементов в организме животных и человека. М., 1953, стр. 61—65.

Волкова А. П. Гиг. и сан., 1959, 1, 80—82.

Волкова И. Н. Физиол. журн. СССР, 1954, 6, 691—696.

Вольфовская Р. Н. и Давыдова Г. Н. Сборник научных работ за годы Отечественной войны. М., 1945, стр. 155—159.

Вредные вещества в промышленности. Под ред. Н. В. Лазарева. Л., 1963, стр. 90—114.

Вялов А. М., Багнова М. Д., Васильев А. С., Пушкина Н. Н. Итоговая научная конференция Московского научно-исследовательского института им. Ф. Ф. Эрисмана, 1961.

Гендерсен, Хагард. В кн.: Вредные газы в промышленности. М.—Л., 1960, стр. 109.

Генес С. Г. Нервная система и внутренняя секреция, 1955.

Гефтер Л. И., Шульман Е. Д., Поляк С. Д., Пильман Н. И., Зарецкая А. И. и Клейн С. М. Труды Воронежского медицинского института, 1935, т. V.

Гиллем А. и Штерн Е. Электронные спектры поглощения органических соединений. Пер. с англ. Под ред. Л. А. Блюменфельда. Изд. Иностранной литературы. М., 1957.

Гирская Е. Я. В кн.: Вопросы гигиены труда, профпатологии и промышленной интоксикации. Свердловск, 1959.

Гительзон Я. Я., Терсков Я. А. Эритрограммы как метод клинического исследования крови. Изд. Сибирского отделения АМН СССР. Красноярск, 1959.

Глауберман С. Б. В трудах Воронежского медицинского института, 1957, т. XXIX, стр. 31—34.

Голщинская М. Т. Физиол. журн. СССР, 1961, XVII, 1, 11.

Голубев А. А. Труды научной сессии Ленинградского института гигиены труда и профзаболеваний, посвященные итогам работы за 1957 г. Л., 1959, стр. 146—153.

Гольдберг М. С. Санитарная охрана атмосферного воздуха. М., 1951.

Гольдберг М. С. Санитарная охрана атмосферного воздуха населенных мест. М., 1960.

Горн Л. Э. Гиг. труда и профзаболевания, 1963, 11, 44—51.

Горшков С. И. О современных методах гигиенических исследований. М., 1964, стр. 9—24.

Гофмеклер В. А. Гиг. и сан., 1960, 4, 9—14.

Гофмеклер В. А. В кн.: Предельно допустимые концентрации атмосферных загрязнений. М., 1961, в. VIII.

Гошев А. И. Вопр. мед. химии, 1958, т. 4.

Григорова О. П. Вопр. охр. мат. и дет. 1953, 10, 50, 55.

Григорова О. П. Чехословацкая педиатрия, 1957, 2, 233.

Григорова О. П., Титов Г. Н. Вопр. охр. мат. и дет., 1957, 1, 31, 34.

Григорова О. П. Вопросы охр. мат. и дет., 1962, 9, 13, 17.

Гришпун Л. Д. Большие эозинофилии крови и их клинико-диагностическое значение. М., 1962.

Гронсберг Е. Ш. Химическая промышленность, 1961, 7.

Гулько Х. Г. Материалы санитарной характеристики производства ванадиевых соединений. Автореф. канд. дисс., 1951.

Гусев М. И. В сб.: Предельно допустимые концентрации атмосферных загрязнений. М., 1960, стр. 7—38.

Гусев М. И. В кн.: Предельно допустимые концентрации атмосферных загрязнений. Медгиз. М., 1960, в. 4. стр. 26.

Гусев М. И. и Смирнов Ю. И. В кн.: Предельно допустимые концентрации атмосферных загрязнений. М., 1960, в. 4, стр. 139—142.

Гусев М. И., Челиканов К. Н. Гиг. и сан., 1963, 5, 3—8.

Дмитриев В. Д. Бюлл. экспер. биол. и мед., 1939, 8, 6.

Дмитриева Н. В. Лабор. дело, 1963, 6.

Егунов А. А. В сб.: Клинико-гигиенические исследования по токсическим веществам, применяемым в новых производствах. Л., 1940, в. 2.

Егоров А. П. Морфологический анализ крови. Медгиз. М., 1954.

Егоров К. В. Труды Астраханского медицинского института, 1958, т. 14, стр. 205.

Еремин Е. П., Каспарская З. А. Биохимия, 1950, т. 15, в. 2.

Жгун П. В. Лабор. дело, 1962, 4, 23.

Жмудская Р. М. Предельно допустимые концентрации ядовитых паров, газов и пыли в воздухе рабочих помещений, 1933, в. 32, стр. 52.

Закржевский Е. Б., Васильева Л. Г. Люминесцентная микроскопия в клинико-гематологических исследованиях. Медгиз. М., 1963.

Имашева Н. Б. Гиг. и сан., 1963, 2, 3—8.

Ицкович А. А., Виноградова В. А. Тезисы докладов научной сессии Новосибирского научно-исследовательского санитарного института 15—18 мая 1966 г. Новосибирск, 1956, стр. 23—26.

Казас Л. И. Архив офтальмологии, 1925, т. 1, стр. 505—526.

Калабухов Н. И. Успехи совр. биол., 1940, 12, в. 1.

Каменский С. С. Материалы для фармакологии ацетофенона. Дисс. докт. СПб, 1889.

Капкаев Э. А. Гиг. и сан., 1963, 12.

Коган А. Х. Бюлл. экспер. биол. и мед., 1959, 4, 8, 10, 109—113.

Коган И. Б. Полярографический анализ в промышленно-санитарной химии. Медгиз. М., 1961, стр. 118—120.

Кожевников В. А., Мещерский Р. М. Современные методы анализа электроэнцефалограмм. Медгиз. М., 1963.

Козлов Н. Б. Автореферат диссертации на соискание ученой степени доктора медицинских наук. Минск, 1962.

Кононова В. А., Аксенова В. Б. Гиг. и сан., 1963, 7, 7—11.

Корбакова А. И., Кремнева С. Н., Кулагина Н. К., Уланова И. П. В кн.: Промышленная токсикология. М., 1960, стр. 232—238.

Короткова Ю. К. Тезисы докладов юбилейной научной сессии института гигиены труда и профзаболеваний АМН СССР, посвященные 40-летию Великой Октябрьской социалистической революции, 16—21 декабря. Ч. III, М., 1957.

Короткова Ю. К. Вестник оториноларинг., 1959, 3, 69—72.

Короткова Ю. К. Промышленная токсикология. М., 1960, стр. 141—150.

Кравков С. В. Физиол. журн. СССР, 1940, 28, 4, 313—322.

Краскина Н. А., Гутрова Н. М. Труды Московского научно-исследовательского института эпидемиологии и микробиологии. Иммунология и профилактика кишечных инфекций. 1962, в. 9, стр. 180—186.

Красовицкая С. Э. Успехи современной биологии, 1951, 32, 2, 166—192. 23 (Д. Я. Шурыгин, С. Ф. Мурчакова, Н. А. Белов, 1957).

Красовицкая М. Л., Запорожец Т. С. В кн.: Материалы научной конференции, посвященной вопросам гигиены труда, профпатологии и промышленной токсикологии в нефтяной и нефтехимической промышленности 30 мая — 2 июня 1961 г. Уфа, 1961, стр. 88—91.

Кратин Ю. Г., Бехтерева Н. П., Гусельников Б. И., Кожевников В. А., Сениченков Б. Т., Усов В. В. Техника и методики электроэнцефалографии. М.—Л., 1963.

Кремнева С. Н. и Толгская М. С. Материалы научной сессии по токсикологии высокомолекулярных соединений. М.—Л., 1961, стр. 60—61.

Кулаков А. Е. Гиг. и сан., 1964, 4, 8—13.

Кулаков А. Е. Гиг. и сан., 1965, 5, 15—20.

Кушаковский О. С. Журн. высш. нерв. деят., 1954, 4, 1, 137—140.

Кырге К. Х. Пробл. эндокринол. и гормонотер., 1956, 2, 4, 110—117.

Лазарев Н. В. Основы промышленной токсикологии, 1938.

Лазарев Н. В. Наркотики. Л., 1940, стр. 188.

Лазарев Н. В. Вредные вещества в промышленности, 1954.

Лазарев Н. В. Вредные вещества в промышленности, 1963.

Ларионов Л. Ф. Гигиена труда и техники безопасности, 1954, 5.

Левин С. И. В кн.: Свердловская областная конференция невропатологов, психиатров и нейрохирургов, 1950.

Левина Э. Н. Сборник работ токсикологической лаборатории института гигиены труда и профзаболеваний, 1948, т. XII, Ленинград.

Лернер И. П., Брусиловский Е. С. Аллергические эозинофильные заболевания. Киев, 1961.

Ли Шэн. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В. VII. М., 1963.

Люблина Е. И. В кн.: Фармакология и токсикология, 1950, 13, 3, стр. 33—37.

Люблина Е. И. В кн.: Исследования в области промышленной токсикологии, 1948, 12, 5, 74.

Магницкий А. Н. Субординация в нервной системе и ее значение в физиологии и патологии. М., 1948.

Макаров П. О. Адекватная оптическая хронаксия в физиологии и клинике. Л., 1952.

Макарченко А. Ф. Изменения нервной системы при интоксикации марганцем. Киев, 1956.

Максимович Я. Б. Журн. высш. нерв. деят., 1954, 4, 6, 909—912.

Мартынова А. П. Вопросы гигиены труда в производстве новых видов синтетических волокон (капронового и анидного). Канд. дисс., 1957.

Мартынова А. П. Гиг. труда и профзаболеваний, 1957, 4, 23—29.

Матанцева Е. И. Гиг. труда и профзаболеваний, 1961, 7, 41—44.

Мейсель М. Н., **Сондакк** В. А. Биофизика, 1956, т. 1, вып. 3, стр. 262—274.

Мерков Л. М. Общая теория и методика санитарно-статистического исследования. Изд. 2-е. М., 1963.

Миллер С. В. В сб.: Предельно допустимые концентрации атмосферных загрязнений. Медгиз, 1955.

Минаев А. А. Сборник трудов конференции молодых гигиенистов и санитарных врачей. М., 1965.

Минкина Н. А. Труды научной сессии Ленинградского научно-исследовательского института гиг. труда и профзаболеваний, посвященной итогам работы за 1956 г. Л., 1958, стр. 237—243.

Мнацаканян А. В. В кн.: Предельно допустимые концентрации атмосферных загрязнений. М., 1964, в. 8, стр. 89—118.

Мурованная С. И. Сборник научных работ Московского областного научно-исследовательского санитарно-гигиенического института. М., 1948, т. 1, стр. 82—124.

Мухитов Б. М. Гиг. и сан., 1962, 6, 16—24.

Мухитов Б. М. В кн.: Предельно допустимые концентрации атмосферных загрязнений. В., VII, М., 1963.

Мытник П. Я. Фармакология и токсикология, 1940, 3, 6, 39—46.

Навроцкий В. К. Вести. АМН СССР, 1960, 3, 57—67.

Назаренко В. А. Труды биогеохимической лаборатории АН СССР, 1937, 4, 265—271.

Некоторые проблемы гигиены труда и профессиональной патологии. Под ред. Э. Р. Мируэдзера. Медгиз. М., 1960.

Николаев Н. М. Сов. мед. 1954, 18, 4, 38.

Нифонтова М. В. В кн.: Методы определения вредных веществ в воздухе и других средах. Ч. I. М., 1960, стр. 238—240.

Новиков Ю. В. В кн.: Предельно допустимые концентрации атмосферных загрязнений, 1957, в. 3, стр. 85—108.

Одошашвили Д. Г. Гиг. и сан., 1962, 4, 3—7.

Олимпиева О. Н., **Ретнер** В. М., **Русинова** А. П. В кн.: Вопросы гигиены труда и профпатологии при работах с бензолом и его гомологами. Институт усовершенствования врачей им. Кирова. Л., 1958, в. 17, стр. 8—22.

Ольшанский М. И. и **Лихачева** В. В. Труды Ростовского-на-Дону медицинского института, 1935, 2, стр. 120—127.
Орлова А. А., **Мазунина** Г. Н. Сов. мед., 1960, 9.
Основы эндокринологии. Под ред. Н. М. Дразнина, М. Ф. Мережинского, 1963.
Павлов И. П. Полное собрание трудов, 1949, т. 5, стр. 112—137 и стр. 142—153.
Пальцев Ю. П. Гиг. и сан., 1964, 2, 28—33.
Пахомычев А. И. Гиг. и сан., 1960, 11, 77—84.
Петров А. А. Успехи химии, 1953, 22, в. 8.
Петров В. И. Гиг. и сан., 1960, 2, 60—62.
Петрунькина А. М. В кн.: Практическая биохимия. М., 1961, стр. 120.
Плотникова М. М. В кн.: Предельно допустимые концентрации атмосферных загрязнений. Под ред. проф. В. А. Рязанова, 1960, в. 4, стр. 75—91.
Покровский А. А. Воен. мед. журн., 1953, 9, 61—65.
Покровский В. А. Токсикология и гигиена производства синтетического каучука. М., 1955.
Покровский В. А. В трудах Воронежского медицинского института, 1957, т. XXIX.
Полетаев М. И., **Андреева** Н. А. Гиг. и сан. 1959, 6, 73.
Попов В. А. В кн.: Материалы Всесоюзной конференции по санитарной охране атмосферного воздуха. М., 1964, стр. 32—33.
Правдин Н. С. О комбинированном действии ядов, 1929.
Предтеченский В. Е. В кн.: Руководство по клиническим лабораторным исследованиям. М., 1960, стр. 249,
Прокопенко Т. А. В кн.: Вопросы гигиены труда, профпатологии и пром. токсикологии, Свердловск, 1958, т. 2, стр. 244—254.
Пушкина Н. Н. В кн.: Биохимические методы исследования. М., 1963, стр. 13.
Рассказова Т. В. Труды Одесского медицинского института им. Н. И. Пирогова, 1958, в. 3, стр. 113—119.
Резник Я. Б. Врач. дело, 1954, 7, 627—631.
Рипп Г. Х. Труды Омского медицинского института, № 48. Омск, 1963.
Рипп Г. Х. Материалы Всесоюзной конференции по санитарной охране атмосферного воздуха, 1964.
Рожкова В. М. Патология, клиника и терапия отравлений. Дисс. Л., 1948.
Розенцвит Г. Э. Рефераты научных работ Ленинградского института гигиены труда за 1953 г. Л., 1954, стр. 148—162.
Ройтбак А. И., **Дедабришвили** Ц. М. Доклады АН СССР, 1959, 124, № 4, стр. 957.
Рощин И. В. Врач. дело, 1952, 9, 819—824.
Рощин И. В. Гиг. и сан., 1952, 11, 49—53.
Рощин И. В. Гиг. труда и профзаболеваний, 1962, 5, 17—22.
Рощин И. В. В кн.: Токсикология редких металлов. М., 1963, стр. 83—95.
Русакова А. В., **Глазова** О. И. Клин. мед., 1943, XXI, 12.
Рязанов В. А. Гиг. и сан., 1961, 6, 3—8.
Рязанов В. А. В кн.: Предельно допустимые концентрации атмосферных загрязнений. Изд. «Медицина», 1964, в. VIII.

Рязнов В. А. Рукозодство по коммунальной гигиене, 1961, т. 1, стр. 158—159.

Рязанов В. А. Гиг. и сан., 1949, 5, 3.

Рязанов В. А. Санитарная охрана атмосферного воздуха, 1951.

Рязанов В. А., Буштуева К. А., Новиков Ю. В. В кн.: Предельно допустимые концентрации атмосферных загрязнений М., 1957, в. 3, стр. 117—152.

Садилова М. С. Материалы XIII научной сессии Свердловского института гигиены труда и профпатологии, 1964.

Садилова М. С. В сб.: Предельно допустимые концентрации атмосферных загрязнений. Медгиз. М., 1957.

Садилова М. С. Материалы VIII научной сессии Свердловского института гигиены труда и профпатологии, 1958.

Садилова М. С. В кн.: Вопросы гигиены труда и профпатологии в цветной металлургии. Свердловск, 1959, т. IV.

Садилова М. С. В кн.: Вопросы гигиены, физиологии труда, профпатологии и промышленной токсикологии. Свердловск, 1962, т. VI.

Садилова М. С. Сборник Уральского филиала Академии Наук СССР, Уральский Государственный университет, 1964.

Селянкина К. П. Гиг. и сан., 1961, 10, 6—12.

Семененко А. Д. Гиг. и сан., 1961, 5, 3.

Семененко А. Д. Гиг. и сан., 1963, 7, 49.

Семененко А. Д. В материалах XIX Московской научно-практической конференции по проблемам промышленной гигиены. М., 1963.

Семененко А. Д. Первая поволжская научная конференция гигиенистов. Куйбышев, 1963, стр. 142—144.

Семененко А. Д. Гиг. и сан., 1963, 7, 49.

Семененко А. Д. и Балашов Б. И. Материалы конференции по итогам научных исследований за 1963 г. Института общей и коммунальной гигиены им. А. Н. Сысина АМН СССР. М., 1964, стр. 34—35.

Сергель О. С., Клименко А. А. Вестник рентгенологии и радиологии, 1957, 5, 76—81.

Серенсен-Роншен. Биохимические методы диагноза и исследования. Мед. изд. Бухарест, 1963, стр. 259.

Сеченов И. М., Павлов И. П., Введенский Е. Е. Физиология нервной системы, 1952, стр. 191.

Соколов Е. Н. В сб.: Ориентировочный рефлекс и ориентировочно-исследовательская деятельность. М., 1958, стр. 11—120.

Слоним А. Д. В кн.: Опыт изучения регуляций физиологических функций. М.—Л., 1953, т. 2.

Соломин Г. И. Гиг. и сан., 1961, 5, 3.

Соломин Г. И. В кн.: Предельно допустимые концентрации атмосферных загрязнений. М., 1962, в. 6.

Соломин Г. И. Гиг. и сан., 1961, 3, 3.

Спыну Е. И. В кн.: Гигиена и токсикология новых пестицидов и клиника отравлений. Медгиз. М., 1962, стр. 257—262.

Стеклова Р. П. В сб.: Ориентировочный рефлекс и ориентировочно-исследовательская деятельность. М., 1958, стр. 183—191.

Стояновский А. Ф. Гиг. и сан., 1961, 10, 70—72.

Стяжкин В. М. В кн.: Предельно допустимые концентрации атмосферных загрязнений. М., 1962, в. 6, стр. 96—103.

Тахиров М. Т. В кн.: Предельно допустимые концентрации атмосферных загрязнений. 1960, в. 4, стр. 39—61.

Тиунов Л. А. и **Денисенко** А. А. Тезисы докладов 5-й Ленинградской конференции по вопросам промышленной токсикологии. Л., 1957, стр. 47.

Ткачев П. Г. Гиг. и сан., 1963, 4, 3.

Тодоров И. Клинические лабораторные исследования в педиатрии. София, 1960, стр. 372—414.

Троп Ф. С. В кн.: Вопросы гигиены труда, профессиональной патологии и токсикологии в промышленности Свердловской области, 1955.

Убайдуллаев Р. В кн.: Предельно допустимые концентрации атмосферных загрязнений. М., 1963, в. VII.

Убайдуллаев Р. Гиг. и сан., 1961, 7, 3.

Уваровская О. М. Проблемы эндокринологии и гормонотерапии, 1956, 2, 3, 110—112.

Уфлянд Ю. М. Теория и практика хронаксиметрии. Л., 1941.

Фаустов А. С. В кн.: Материалы научной конференции, посвященной вопросам гигиены труда, профпатологии и промышленной токсикологии в нефтяной и нефтехимической промышленности. Уфа, 1961, стр. 165—167.

Фельдман Ю. Г. В кн.: Предельно допустимые концентрации атмосферных загрязнений. М., 1962, в. 6, стр. 109—127.

Фомин А. П. Сборник трудов конференции молодых гигиенистов и санитарных врачей. М., 1965.

Фотонич И. Д. В кн.: Производство азотных удобрений. М., 1956.

Фрейдлин В. С. Труды Воронежского медицинского института и областной санитарно-гигиенической лаборатории Воронежского облздравотдела, 1935, т. 5.

Ходас М. Я. Мед. радиол., 1959, 3, 44—43.

Черниговский В. Н., **Ярошевский** А. Я. Вопросы нервной регуляции системы крови. Медгиз. М., 1953.

Чернов В. М. Фармакол. и токсикол., 1947, 2, X, 39—41.

Чжао Чжэн-ци. Гиг. и сан., 1959, 10, 7—12.

Чижиков В. А. Гиг. и сан., 1963, 6, 8—15.

Чижиков В. А. В кн.: Предельно допустимые концентрации атмосферных загрязнений. М., 1964, в. 8, стр. 21—41.

Чирков С. К. Аналитическая химия, 1957, т. 12, в. 6.

Чэнь Юнь-тай. Гиг. и сан., 1963, 2, 93—95.

Штессель Т. А. Физиол. журн. СССР, 1937, 22, 1, 129—137.

Эскин И. А., **Видавская** Г. М. Проблемы эндокринологии и гормонотерапии, 1956, 11, 1, 82—87.

Эскин И. А., **Михайлов** Н. В. Бюлл. экспер. биол. и мед., 1958, 46, 8, 100—104.

Ярославская Р. И., **Розовский** И. М. В кн.: Второе Всесоюзное совещание по вопросам промышленной токсикологии 4—6 февраля 1952 г. М., 1952, стр. 12—13.

б) *Иностранная*

Amdur M. O., **Mead** I. The respirotary respons of guinea pigs to inhalation of ethylene oxide. A. M. A. Archives of industrial Health., 1956, 14, 553.

Aschkenasy A. Le rôle des hormones dans la régulation de l'eosinophilie sanquine. Sanq, 1957, 5, 400.

Gessner P. K., Parke D. V., Williams R. T. Biochem. J., 1961, 79, 3.

Cholak и др. Arch. of Industrial Hygiene and Occupational Medic., 6, 4.

Cristiani H. Le fluor des os dans l'intoxication fluorique. Ann Hyg. Pub. Ind. et Sociale, 1930, 8, 309—316.

De Eds F. Fluorine relation to bon and tooth development, J. Am. Dental Assoc., 1936, 23, 563—574.

Dohan F. C. Arch Environomental Health, 1961, 3, 4, 387—395.

Drabkin D. L., Austin J H. J. Biol. Chem., 1935—36, 112—31.

Dutton W. J. A. M. A., 1911, 56, 1648.

Phillips P. H. Plasma phosphatase in diary cows suffering from fiuorosis. Science, 1932, 76, 239—40.

Franke, Moxson J. Pharm. (Lond.), 1936, 58, 4, 454; 1937, 61 1, 89.

Friebel H., Gross E., Immisch-Seehausen L., Linke K u. Sommer S. Zur Toxicität von reinem Phthalsäreahyorid und Rohrproducten aus der industriallen Phthalsäresynthes. Arch. für Gewerbepathologie und Gewerbehygiene, 1956. Bd. 14, s. 465—482.

Friedman E., Maase C. Zur Kenntnis des Abbaner der Karbonsaren im Tierkörper. Biochem. J., 1910, 27, 111—114.

Harold M., Nielsen, Logan, Utan. The determination of fluorine in soft tissues, bone and urine. Arch. Industr. Health, 1960, v. 21, 4.

Henderson, Haggaren. Вредные газы в промышленности. М—Л., 1930, Пер. с англ.

Hestrin S. J. biol. Chem., 1951, 180, 249.

Hollingsworth R. L., Rowe V. K., Oyen F., McCollister D. D. Toxicity of ethylene oxide determined on experimental animals. A.A.A. Arch. Industrial Health., 1956, 13, 3, 217.

Horvath. Japan Med. World. 6, No 2, 1926; Proc. Soc. Expl. Biol. a. Med. 22, 199, 1929.

Granati A., Scavo D. Il ricambio protidico nel Sulfocarbonismo cronico. Folia. Med., 1956, 39, 8, 763—774.

Jacobson K. H., Hackley E. B. The toxicity of inhaled ethylene oxide and pripylene oxide vapors. A.M.A. Arch. of Industrial Health., 1956, 13, 3, 237.

Kapalin V. Čs. hyg., 1958, 3, 386.

Kapalin V. Čs. pediatr., 1961, 19, 7, 646.

Kapalin V., Janáčková H. Čs. pediatr., 1961, 19, 2, 171.

Kapalin V., Procopec M., Prosec J. Cs. pediatr., 1957, 12, 420.

Kerr A. C. The effect of mental stress on the eosinophil leucocyte count in man. Quart. J. exp. Physiol., 1956, 1, 18.

Laborde J. V. Note sur l'action phys ologique et toxique de l'acetophenone et phenilmethylacetone. Compt. rend. Sol. de biol. Par., 1885, 8, pt., 2, 725—737.

Levy R. J. J.A.M.A., 1960, 173, 11.

Lewis C. E. Arch. hel Health, 1959, 20, 445—446

Lilly J. C., Hughes J. R., Alvord E. G., Galvin T. W. Fed. Proc., 1955, 14, 93.

Maglior Mario, Es de Rosa Carlo. Il quadrosiero proteico del conglio nell'intossicazione naftalinica. Ann. Oftalmole Chin. Ocul., 1957, 83, 9, 438—442.

Marhold J. Přehled peumislové toxikologie. Stat. zdrav. nakl. Praha, 1964.

Mitchell Proc. Soc. exp. Biol. (N. Y.), 1953, 84, 2, 404—405.

Molfino. Ber. üb. d. ges. Physiol., 1939, 114, 173.

Morette A. Ann. pharmac. franç., 1957, 15, 4, 209—216.

Murphy J. P., Gellhorn E. J. Neurophysiol., 1945, 8, 337.

Nischiama. Isami-Studies on the benzol poisoning. Part II. Poisoning by mixture of benzol with acetone or butylacetate. S. Sci. Labor., 1957, 33, 12, 969, 997.

Paton W. Histamine release by compound of simple chemical structure. Pharmacological Reviews, 1957, 9, 2, 269—328.

Petr B. Problemazika znečištění ovzduší v okolí závodu, Sborník ÚHPCHP. Praha, 1963.

Recine H. Med. a. Zovaro., 1956, 47, 5, 346—349; 1956, 47, 3, 172—175, 167—171.

Roholm K. Fluorine intoxication. London, 1937.

Roholm K. Serum phosphatase activity in generalized osteosclerosis in man. Proc. Soc. exp. Biol. (N. Y.), 1937, 37, 376—9.

Rosenbaum E. J., Stanton L. Continous recording ultraviolet spectophotometer. Anal. Chem., 1947, 19, 794.

Watson C. J. The porphyrens and deseases of the blood. Sympos. on the blood and blood forming organs. Machson 1941, 14—30.

Schmidt P., Knotek Z., Musil J. Čs. pediatr., 1960, 18, 9.

Scholz J. Klin. Wschr., 1950, 3—4.

Silverman L., Whittenberger J. Z. a. Muller J. J. Industrial. Hyg. a. Toxicol., 1949, 31, 2, 74—78.

Smith M. C., Lanz E. M. The effect of fluorine upon the phosphatase content of plasma, bones and teeth of albin rats. J. biol. Chem., 1935, 112, 303—11.

Snyder F., Cornatzer W. Natura, 1958, 182, 4633, 462.

Symanski Arch. Gewebepath. u. Gewebehyg., 1939, 9, 295.

Symon K. a. j. Čs. hyg., 1960, 5, 2/3, 88.

Symon K. Čs. hyg., 1964, 9, 8, 475.

Thorn G. W. Nebenniereninsuffizienz Diagnose und Behandlung. Bern-Stuttgart, 1953.

Tomas A. J. publ. Hlth., 1959, 49, 12, 1664—69.

Thomas F. E., Davidson C. H. J. Air. Poll. Contr. Assoc., 1961, 11, 24, 27.

Thomas J. O., Wilson R. H., De Eds T. Bone phosphatase activity in chronic fluorine poisoning. J. Pharmacol. exp. Ther., 1935, 54, 160.

Joya Ayo. В реферат. журн. «Биология», 1959, 21, 327.

Troisi F. M. Brit. J. Ind. Med., 1950, 7, 2, 65—69.

Trummert, Boehm, Blut., 1957, 3, 4, 211—216.

Ubl Z. Acta hyg., Příloha č. 2, 1964.

Waite C. P. Scute response of guinea pigs to vapors of some new commercial organic compounds: IV ethylene oxide. Public Health Repts., 1930, 45, 31, 1832.

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